

1/254

FIGURE 1

Complete Sequence of Baculovirus Transfer Vector, pVL1393

ID PVL1393 preliminary; circular DNA; SYN;
9632 BP.
XX
AC IG1137;
XX
DT 01-FEB-1993 (Rel. 7, Created)
DT 01-JUL-1995 (Rel. 12, Last updated, Version
1)
XX
DE E. coli plasmid vector pVL1393 - complete.
XX
KW cloning vector.
XX
OS Cloning vector
OC Artificial sequences; Cloning vehicles.
XX
RN [1]
RC p2Bac from baculovirus
RC p2Blue from p2Bac
RC pBlueBac from AcNPV
RC pBlueBac2 from AcNPV
RC pBlueBacIII from AcNPV
RC pBlueBacHisA from AcNPV
RC pBlueBacHisB from AcNPV
RC pBlueBacHisC from AcNPV
RC pVL1392, pVL1393 from pAc360
RA ;
RT ;
RL The Digest 5:2-2(1992).
XX
CC NM (pVL1393)
CC CM (yes)
CC NA (ds-DNA)
CC TP (circular)
CC ST ()
CC TY (plasmid)
CC SP (British
Biotechnology) (Invitrogen)
CC HO (E.coli NM522) (E.coli
INValphaF') (insect)
CC CP ()
CC FN (expression) (transfer)
CC SE ()
CC PA (pAC360)
CC BR (pVL1392)
CC OF ()
CC OR ()
XX
FH Key Location/Qualifiers
FH

004400"TESTS60

FIGURE 1 (Cont'd)

```
FT      misc_feature          0..0  
FT                                     /note="1. pAc360, ori/amp/AcMNPV  
polyhedrin gene  
FT                                     -> pVL1393 9632bp"  
FT      transposon           0..0  
FT                                     /note="TRN AcMNPV"  
FT      misc_binding         868..868  
FT                                     /note="SIT SacII"  
FT      misc_binding         1395..1395  
FT                                     /note="SIT ApaI"  
FT      misc_binding         1901..1901  
FT                                     /note="SIT XhoI"  
FT      promoter             0..0  
FT                                     /note="PRO AcMNPV polyhedrin gene"  
FT      misc_binding         0..0  
FT                                     /note="MCS  
FT                                     BamHI-SmaI-XbaI-EcoRI-NotI-XmaIII-PstI-  
BglIII"  
FT      rep_origin           0..0  
FT                                     /note="ORI E. coli pMB1 (ColE1 and  
pBR322)"  
FT      CDS                  complement(0..0)  
FT                                     /note="ANT E. coli beta-lactamase gene  
(bla)"  
FT      ampicillin resistance gene (apr/amp)"
```

SQ Sequence 9632 BP; 2602 A; 2122 C; 2176 G; 2732 T; 0
other;

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gcggggcctgt	tatacaaaaa	aattcaagta	cctggccaga	ctttgcgcgc
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tggccggtaa	tagccgtaaa	cgtagtgccg	tcgcgcgtca	cgcacaacac
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FIGURE 1 (Cont'd)

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FIGURE 1 (Cont'd)

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FIGURE 1 (Cont'd)

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6/254

FIGURE 1 (Cont'd)

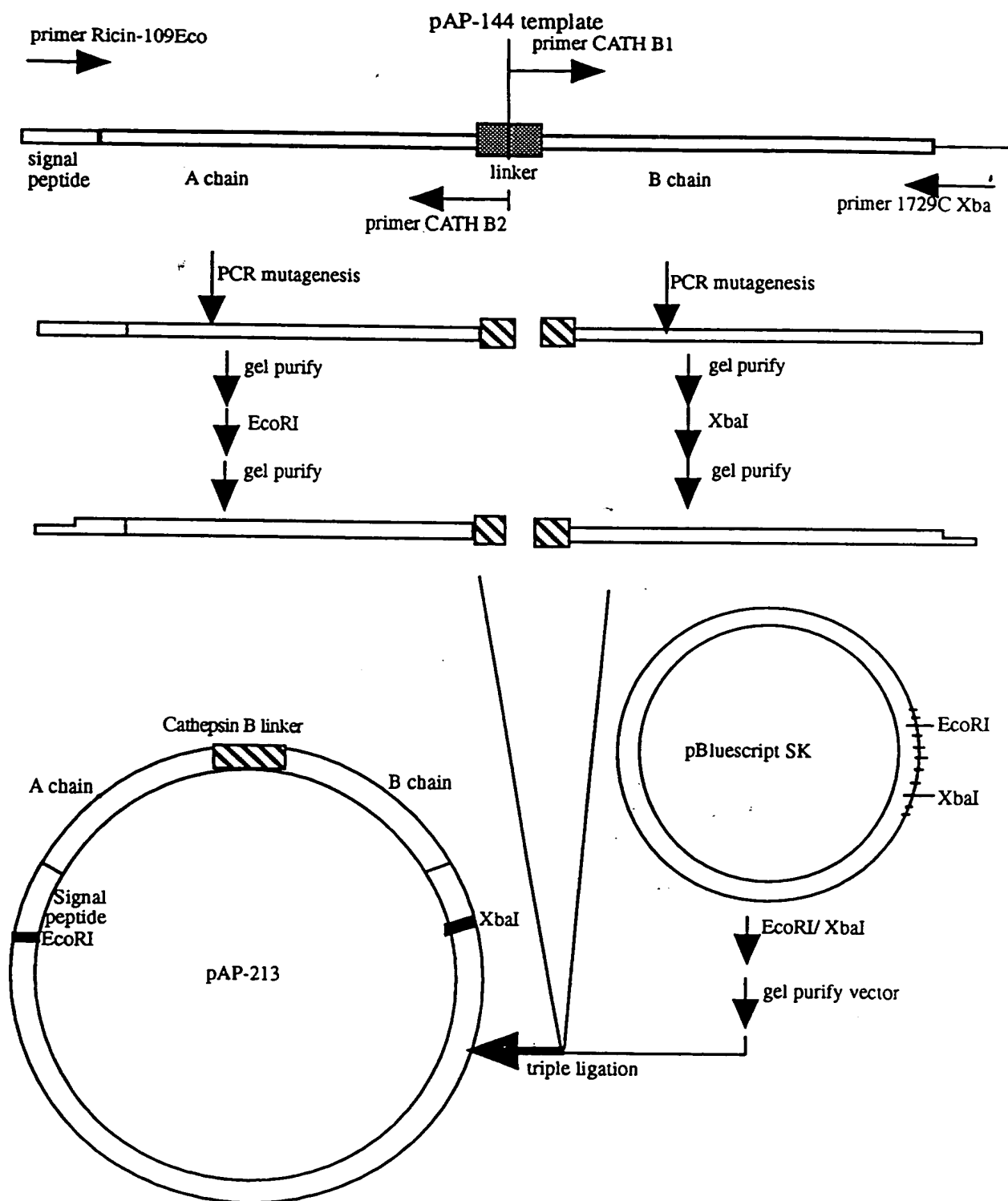
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gggttttccc agtcacgacg ttgtaaaacg acggccagtg cc

//

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7/254

FIGURE 2A



0955151-041400

FIGURE 2B

WT preprorin linker

primer CATH-B1

5'- ATGGTGCCAAATTTAAT-3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTAAT
AGAACGGAATATTCGGTCACCCAGGTTAAATA

3'-TCTCGATTTAAGCAAGAAACTG-5'

primer CATH-B2

PCR mutagenesis

ligate with pBluescript SK

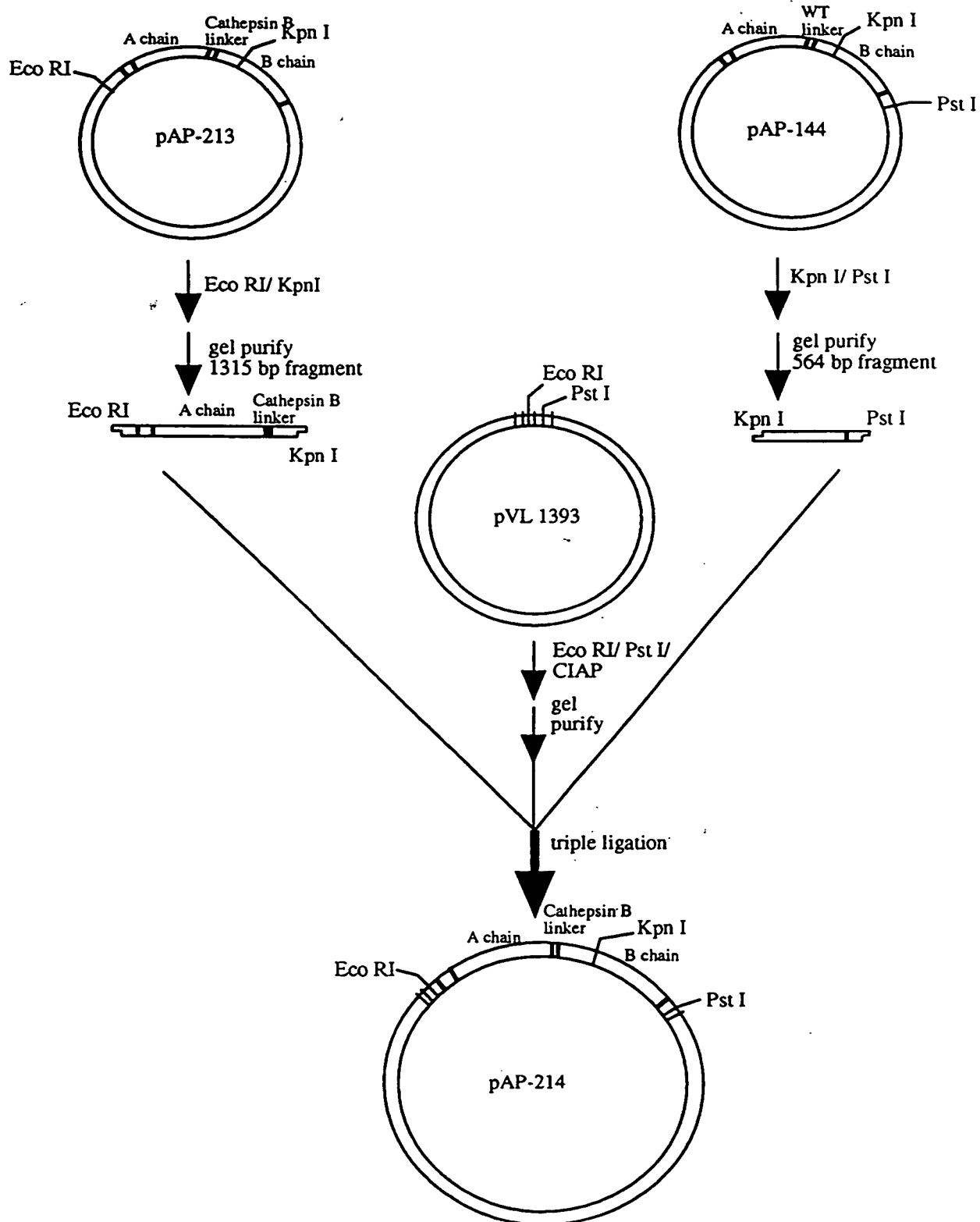
pAP 213 linker

(Cathepsin-B variant)

TCTTTGCTTAAATCGAGAAATGGTGCCAAATTTAAT
AGAACGGAATTTAGCTCTTACCAGGTTAAATA

9/254

FIGURE 2C



0951151-041400

10/254

FIGURE 2D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTCCGCG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAATAAAGTTTAA

451 CGATATACATTTCGCTTTTGGTGGTAATTATGATAGACTTGAACAACCTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
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751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
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801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTGGT

901 TCGTCACAGTTTTCTTTGCTTAAATCGAGAATGGTGCCAAATTTTAATGC
AGCAGTGTCAAAGAAACGAATTTAGCTCTTACCACGGTTTAAATTTACG

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FIGURE 2D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCCTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

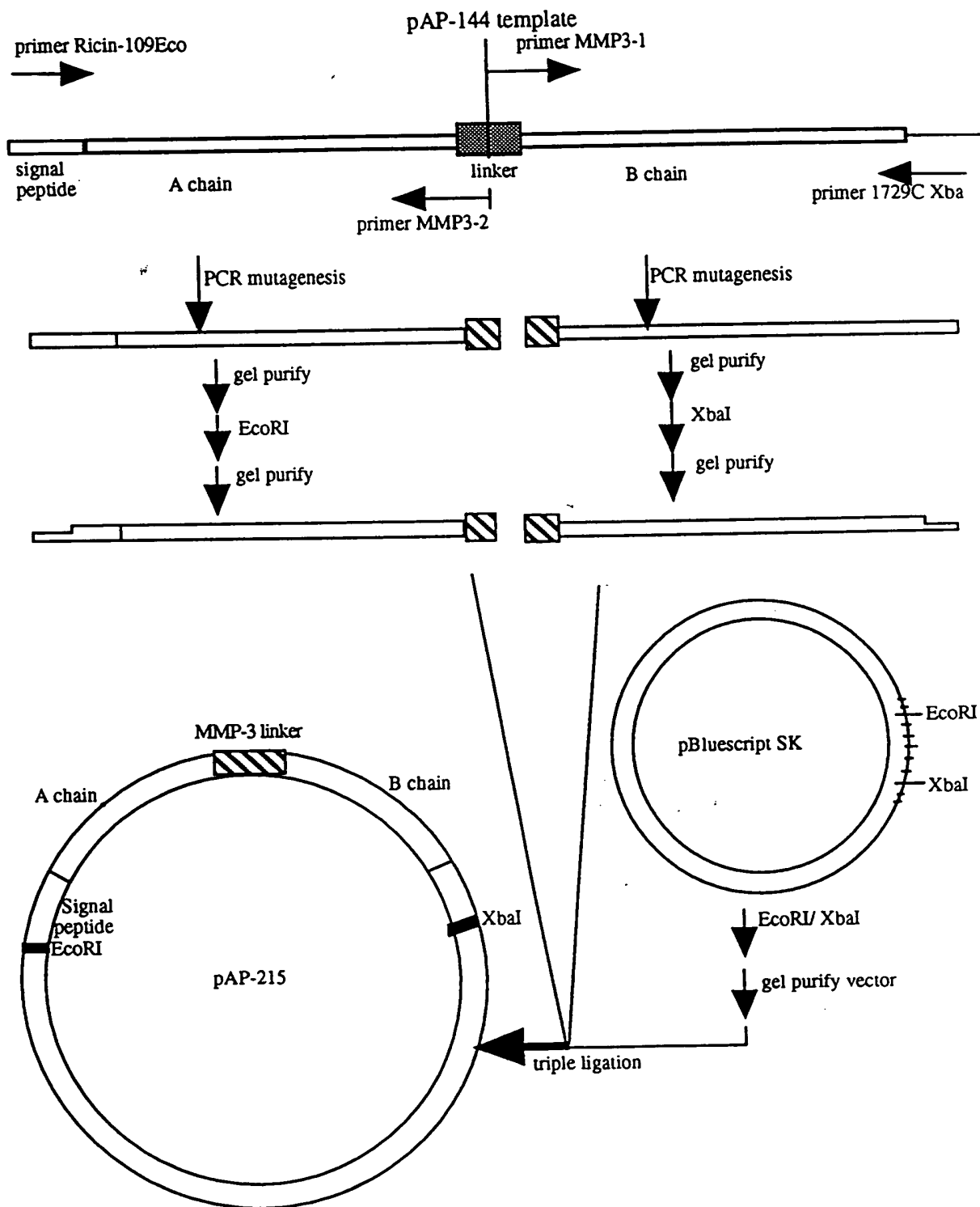
1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTGAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

12/254

FIGURE 3A



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FIGURE 3B

WT preprorin linker

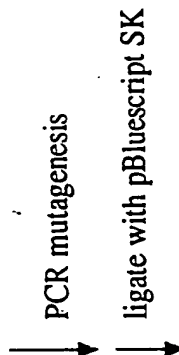
primer MMP3-1

5' - TTTTGGACTTATGAATGCTGATGTTGT -3'

3' - TCTTGCTTATAAGGCCAGTGGTGCCCAATTTTAAT
AGAAACGAATATTCGGTCACCCACGGTTTAAATTA

3' - GGTAGCAGTGTCAAAGCAGGCTTCGGTGTGTT -5'

primer MMP3-2

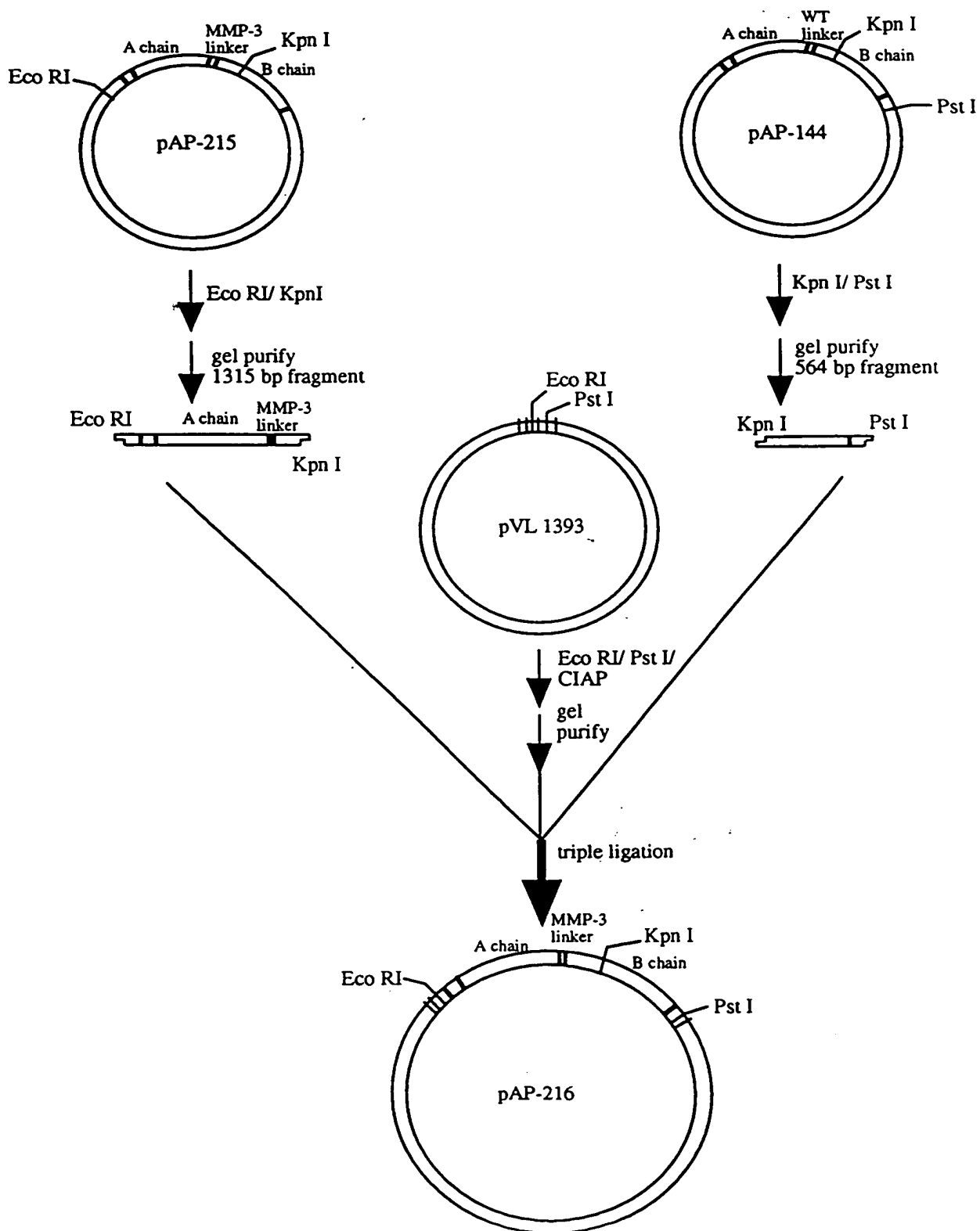


pAP 215 linker
(MMP-3 variant)

CGTCCGAAGCCACAGCAATTTTGGACTTATGAAT
GCAGGCTTCGGTGTGTTAAACCTGAATACTTA

14/254

FIGURE 3C



00440" TSTT5560

FIGURE 3D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC

101 AGGATAACAACATATTCCTCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTTCCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAATAGCGCATATTTCTTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAATAAAGTTT

451 CGATATACATTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC
GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTGAAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCCTTTATAATTTGCATCCAAATGATTTTCAAGAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTGGT

901 TCGTCACAGTTTCGTCCGAAGCCACAGCAATTTTTTGGACTTATGAATGC
AGCAGTGTCAAAGCAGGCTTCGGTGTGCTTAAAAACCTGAATACTTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTGCAATG

FIGURE 3D (CONT'D)

ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAGATTATGCTACGTTTAGTCGAGACCTGAAA
1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTACAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCTATTACCTTGGTAGTATTTAGG
1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351 AATAATACACAACCTTTTGTACAAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT
1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501 CAAAACCGAGATAATTGCCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA
1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCCTTACTACCTTGGTAAAAATTTAAACATATCACCTAACCACAATCTA
1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA
1751 CTCTTGAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851 TGCAG
ACGTC

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17/254

FIGURE 4A

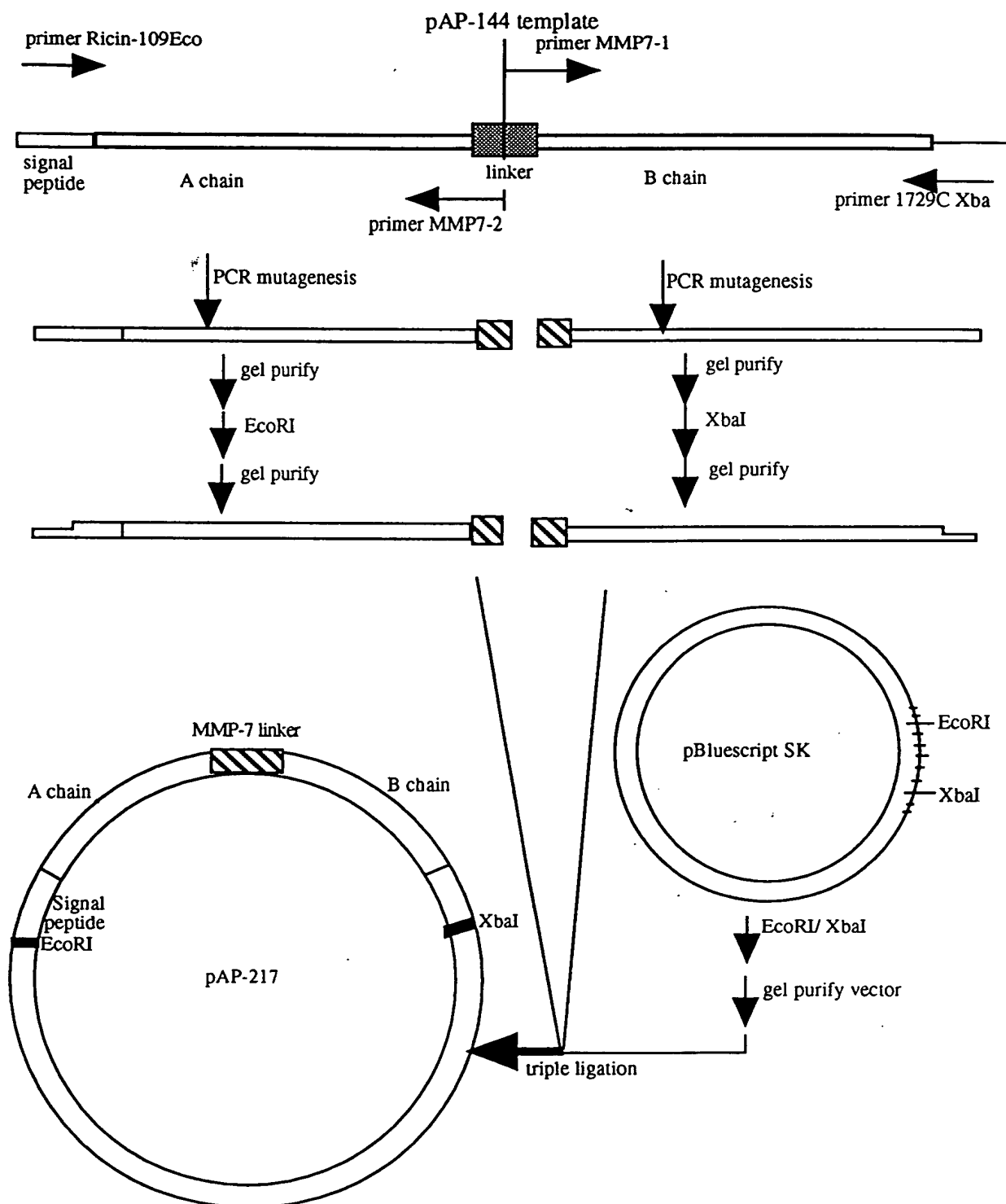


FIGURE 4B

WT preproricin linker

primer MMP7-1

5' - TTGTGGCGAAGTTTAAATGCTGATGTT-3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTAAAT
AGAAACGAATATTCCTGGTCAACCGGTTTAAATTA

3' - AGTGTCAAAAGAAACGACGGTGACCGT-5'

primer MMP7-2

18/254

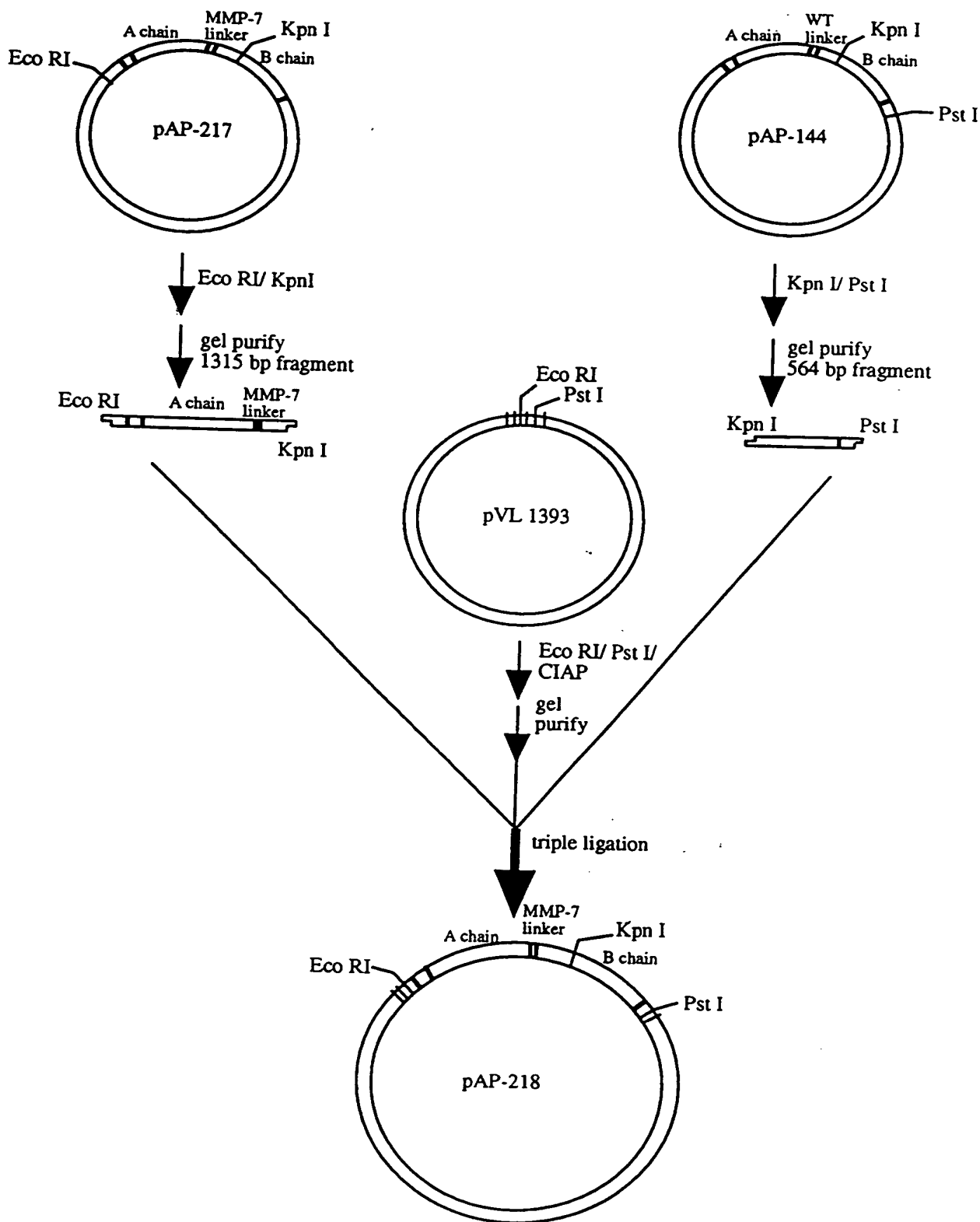
PCR mutagenesis
ligate with pBluescript SK

pAP 217 linker
(MMP-7 variant)

TCTTTGCGTCCACTGGCATTTGTGGCGAAGTTTAAAT
AGAAACGCAGGTGACCGTAACACCGCTTCAAAATTA

19/254

FIGURE 4C



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FIGURE 4D

10 20 30 40 50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAATAAAGTTTGA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTGAAACG
501 TGGTAATCTGAGAGAAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCCTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCTGTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTCTCAGATTGGTTCCCTCGGAAACGATCAGGTTA
801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTGGT
901 TCGTCACAGTTTCTTTGCGTCCACTGGCATTGTGGCGAAGTTTTAATGC
AGCAGTGTCAAAGAAACGAGGTGACCGTAACACCGCTTCAAATACG
951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTGAAATG

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FIGURE 4D (CONT'D)

ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

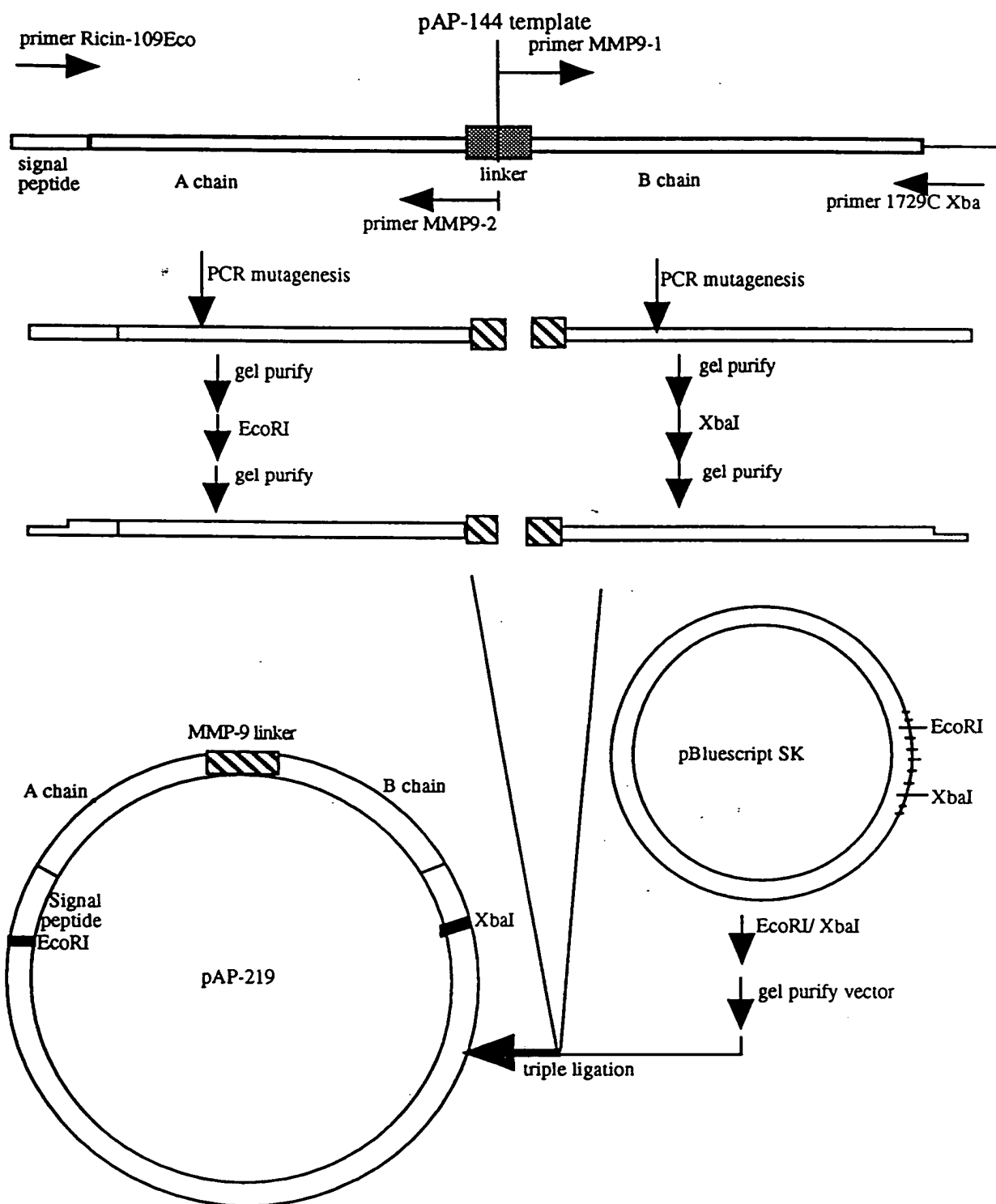
1701 TGGTGACCCAAACCAAATATGGTTACCATTTATTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCTCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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FIGURE 5A

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FIGURE 5B

WT preprorincin linker

primer MMP9-1

5' - GGGCAGCGAAATTTTAATGCTGAT -3'

TCTTTCGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCTCGGTACCCACGGTTTAAATA

3' - AGCAGTGTCAAAAGAGCGGTCCTTAACGT -5'

primer MMP9-1

PCR mutagenesis

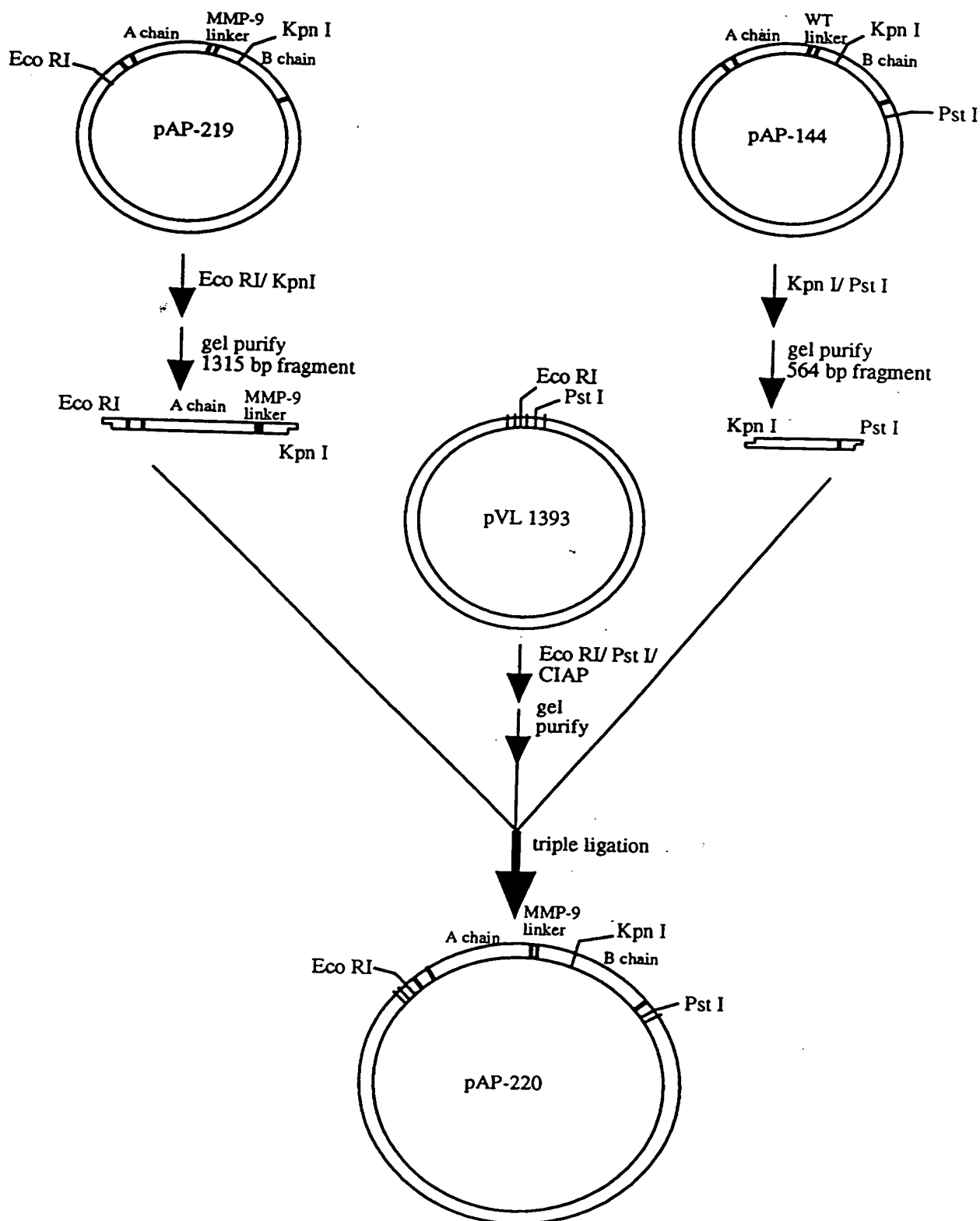
ligate with pBluescript SK

pAP 219 linker
(MMP-9 variant)

TCTCCGCAAGGAATTCAGGCGCAGCGAAATTTTAAT
AGAGCGGTCCTTAACGTCCCGCTTAAATA

24/254

FIGURE 5C



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FIGURE 5D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTT

451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTGTAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTCTCCGCAAGGAATTGCAGGGCAGCGAAATTTTAATGC
AGCAGTGTCAAAAGAGGCGTTCCTTAACGTCCCGTCGCTTTAAATTACG

FIGURE 5D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTTACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTACACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

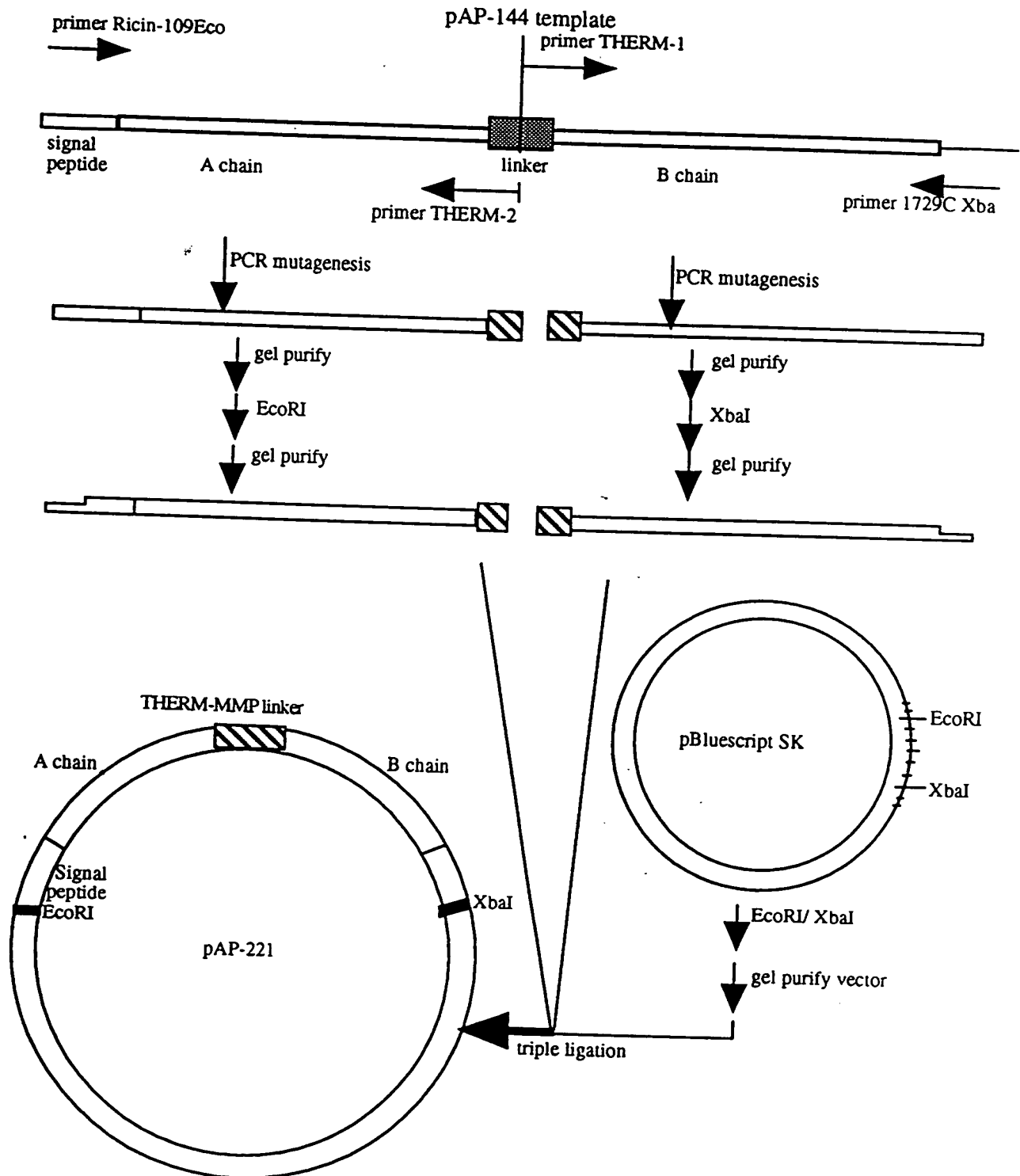
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

0951151 "041400

27/254

FIGURE 6A



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FIGURE 6B

WT preprorin linker

primer THERM-1

5' - AGGGAATTTTGCTTCTTTTGTAGTGATGTTGTATG - 3'

*** ** ** ** **
 TCTTTGCTTATAAGGCCAGTGTGCGCAATTTTAAT
 AGAAACGAATATTCCTGGTCAACACCGTTTAAATTA
 *** ** ** **

3' - GGTGGTAGCAGTGTCAAACTACACCTACTTCCCTACAC - 5'

primer THERM-2

PCR mutagenesis

ligate with pBluescript SK

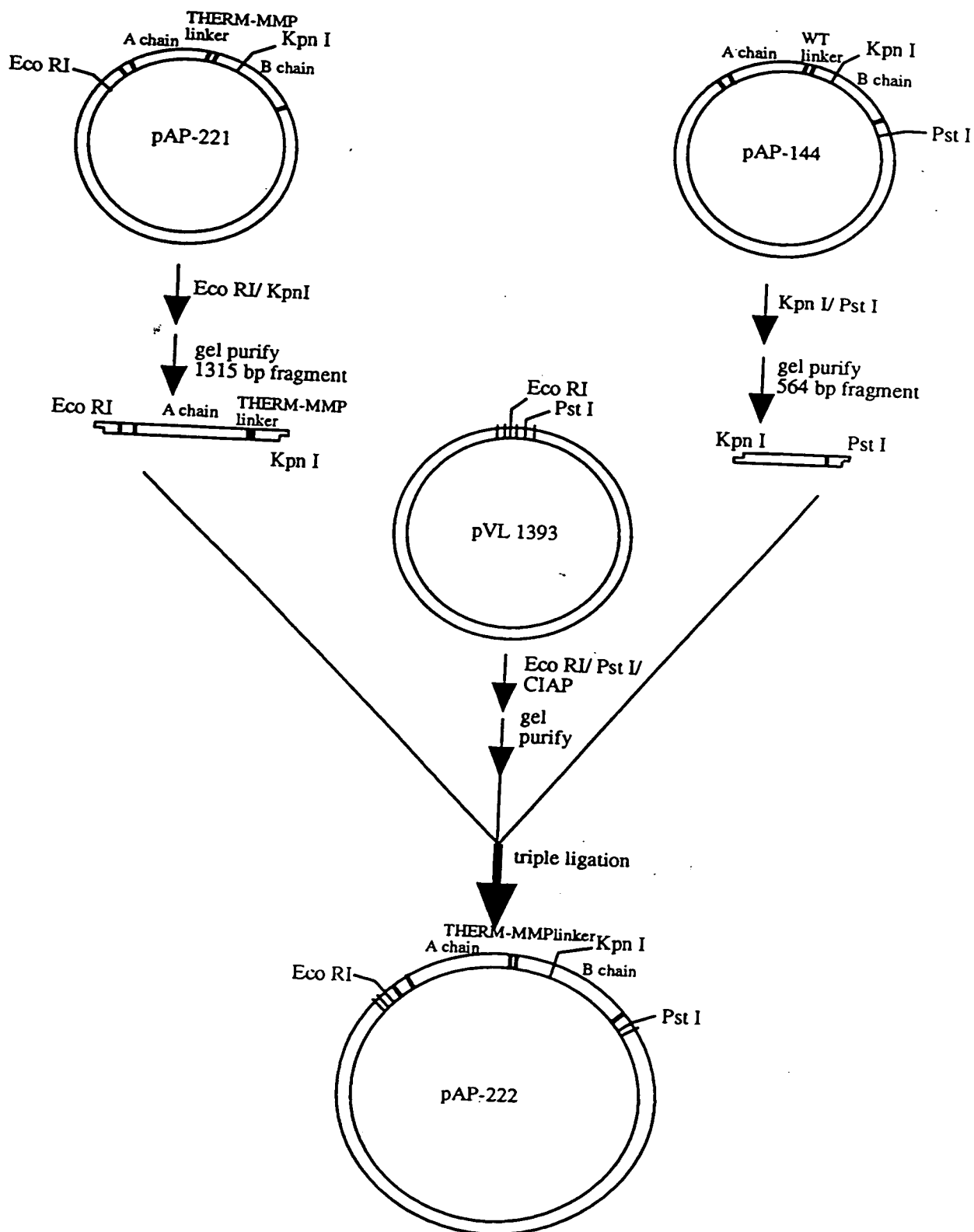
New Cleavage Site

pAP 221 linker
 (THERM-MMP variant)

GATGTGGATGAAAGGATGTGAGGGAATTTGCTTCTTTTA
 CTACACCTACTTCCCTACACTCCCTTAAACGAAGAAAAAT

29/254

FIGURE 6C



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30/254

FIGURE 6D

	10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA				
51	GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG				
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC				
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
	TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT				
151	GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG				
	CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC				
201	TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA				
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251	ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA				
	TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT				
301	AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351	TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTTCATCCTGACA				
	ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT				
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT				
	TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTPTA				
451	CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC				
	GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTGTTGAACG				
501	TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC				
551	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601	CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAGAAGCAGCAAG				
	GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT				
651	ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT				
701	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA				
	CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTPATCAACCCCCCTCT				
751	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT				
	GAAAGGTGACGTTAAGTTCTCAGATTGGTTCTCGGAAACGATCAGGTTA				
801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA				
	AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT				
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT				
901	TCGTCAAGTTTGTATGTGGATGAAAGGGATGTGAGGGAATTTGCTTCTTT				
	AGCAGTGTCAAACCTACACCTACTTCCCTACACTCCCTTAAACGAAGAAA				
951	TTTAGCTGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTC				

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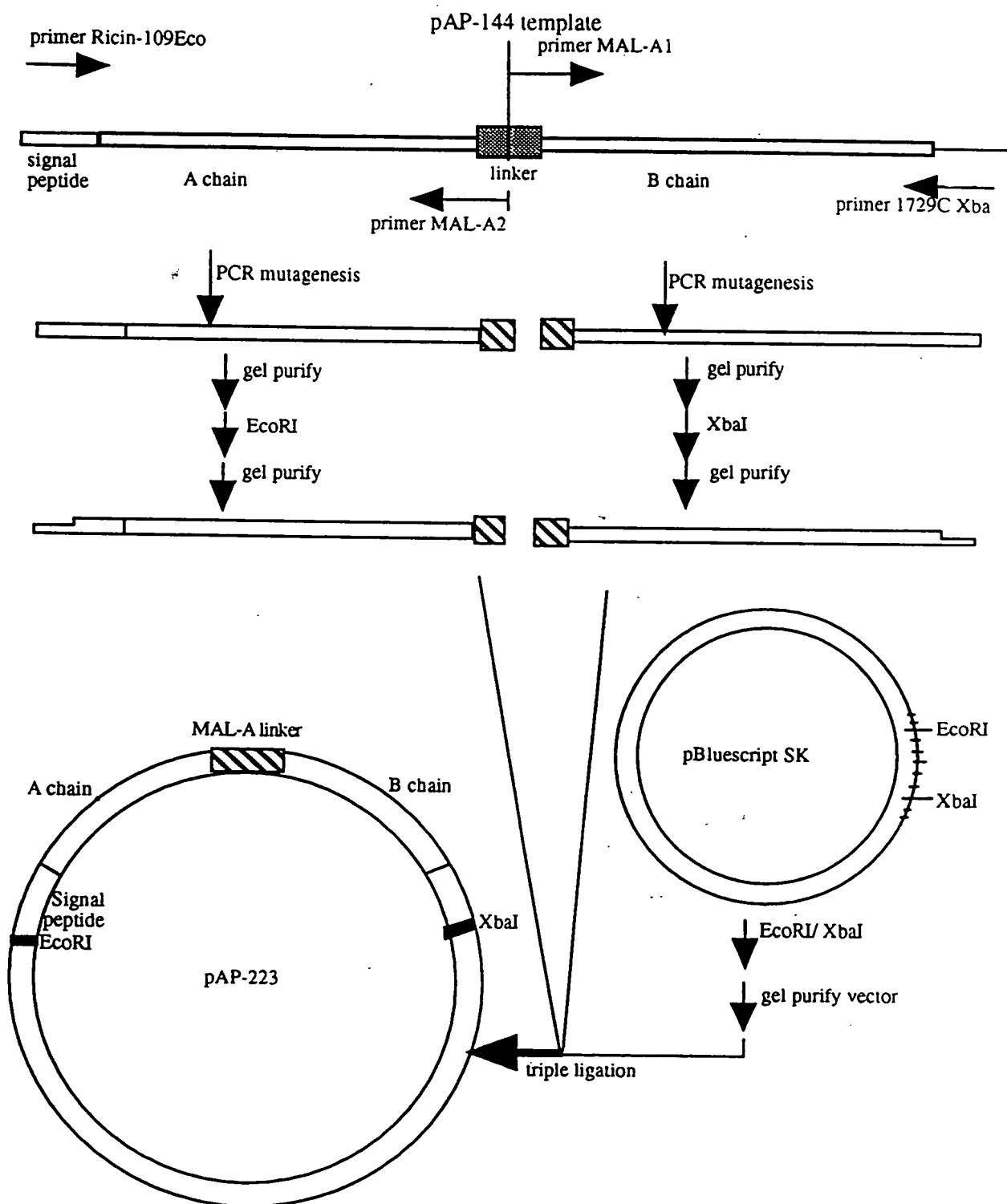
FIGURE 6D (CONT'D)

AAATCGACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAG
1001 GAAATGGTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAAC
CTTTACCAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTG
1051 GCAATACAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTG
CGTTATGTCAACACCGGTACGTTTCAGATTATGTCTACGTTTAGTCGAGAC
1101 GACTTTGAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTA
CTGAAACTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGAT
1151 CTTACGGGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACT
GAATGCCCATGTACGGCCCTCAGATACACTACTAGATACTAACGTTATGA
1201 GCTGCAACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCAT
CGACGTTGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTA
1251 AAATCCCAGATCTAGTCTAGTTTGTAGCAGCGACATCAGGGAACAGTGGTA
TTTAGGGTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCAT
1301 CCACACTTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTGGCTT
GGTGTGAATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAA
1351 CCTACTAATAATACACAACCTTTTGTACAAACCATTGTTGGGCTATATGG
GGATGATTATTATGTGTTGGAACAATGTTGGTAACAACCCGATATACC
1401 TCTGTGCTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCA
AGACACGAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGT
1451 GTGAAAAGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGT
CACTTTTCCGACTTGTGTACCCGAGAAATACGTCTACCAAGTTATGCA
1501 CCTCAGCAAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGA
GGAGTCGTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCT
1551 AACAGTTGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGAT
TTGTCAACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTA
1601 GGATGTTCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTG
CCTACAAGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCAC
1651 TTAGATGTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCC
AATCTACACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGG
1701 TCTCCATGGTGACCCAAACCAAAATATGGTTACCATTATTTTGATAGACAG
AGAGGTACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCT
1751 ATTACTCTCTTGCAAGTGTGTGTCTGTCCTGCCATGAAAATAGATGGCTTAAA
TAATGAGAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTT
1801 TAAAAAGGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCG
ATTTTTCTGTAAACATTTAAACATTGACTTTCCTGTCTCGTTCAATATAGC
1851 AATTCCTGCAG
TTAAGGACGTC

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32/254

FIGURE 7A



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FIGURE 7B

WT preprorin linker

primer MAL-A1

5' - AATTATGATGAAGAGGATGCTGATGTTTGTATG -3'
 |*****
 TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
 AGAAACGAAATATTCCGGTCACCCACGGTTTAAATTA

 3' - GGTAGCAGTGTCAAAGTCCACCAAGTTAACGTC -5'

primer MAL-A2

PCR mutagenesis

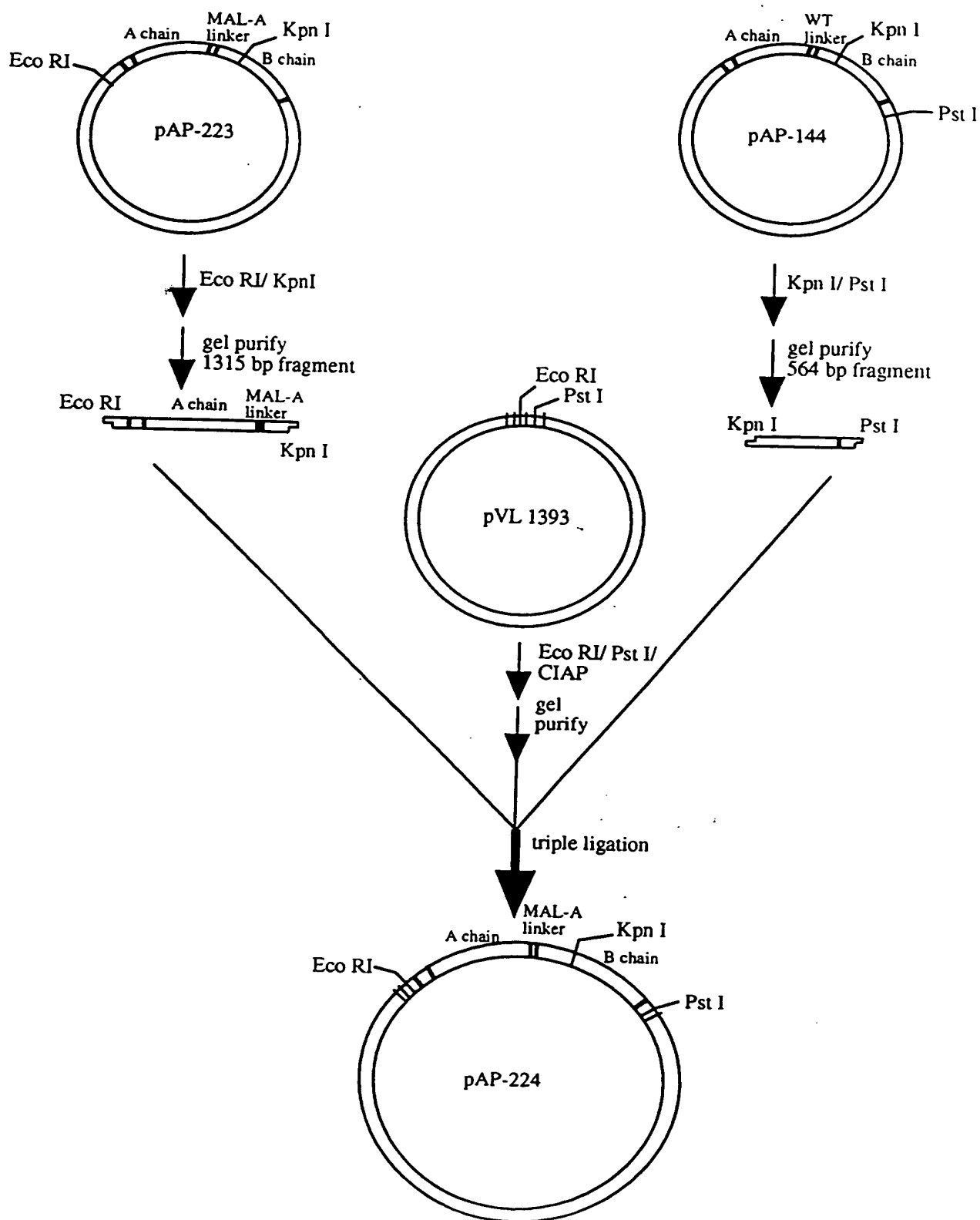
ligate with pBluescript SK

pAP 223 linker
 (MAL-A variant)

CAGGTGGTTCAATTCCAGAATTATGATGAAGAGGAT
 GTCCACCAAGTTAACGTCTTAATACTACTTCTCCTA

34/254

FIGURE 7C



0955151-041400

35/254

FIGURE 7D

	10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA				
51	GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG				
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC				
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
	TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT				
151	GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG				
	CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC				
201	TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA				
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251	ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA				
	TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT				
301	AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351	TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA				
	ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT				
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT				
	TAGTCCTTCTACGTCTTCGTAGTGAGTAGAAAAGTGACTACAAGTTTTA				
451	CGATATACATTTCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC				
	GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACCTGTTGAACG				
501	TGGTAATCTGAGAGAAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC				
551	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601	CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG				
	GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT				
651	ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT				
701	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA				
	CTAGACGTGGTCTAGGATCGCATTAAATGTGAACCTTTATCAACCCCTCT				
751	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT				
	GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA				
801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA				
	AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT				
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT				
901	TCGTACACAGTTTCAGGTGGTTCAATTGCAGAAATTATGATGAAGAGGATGC				
	AGCAGTGTCAAAGTCCACCAAGTTAACGTCTTAATACTACTTCTCCTACG				

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FIGURE 7D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAGATTATGTCTACGTTTTCAGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTGAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTTCGCTGTAGTCCCTTGTCAACATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACCAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTACCCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTCTCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

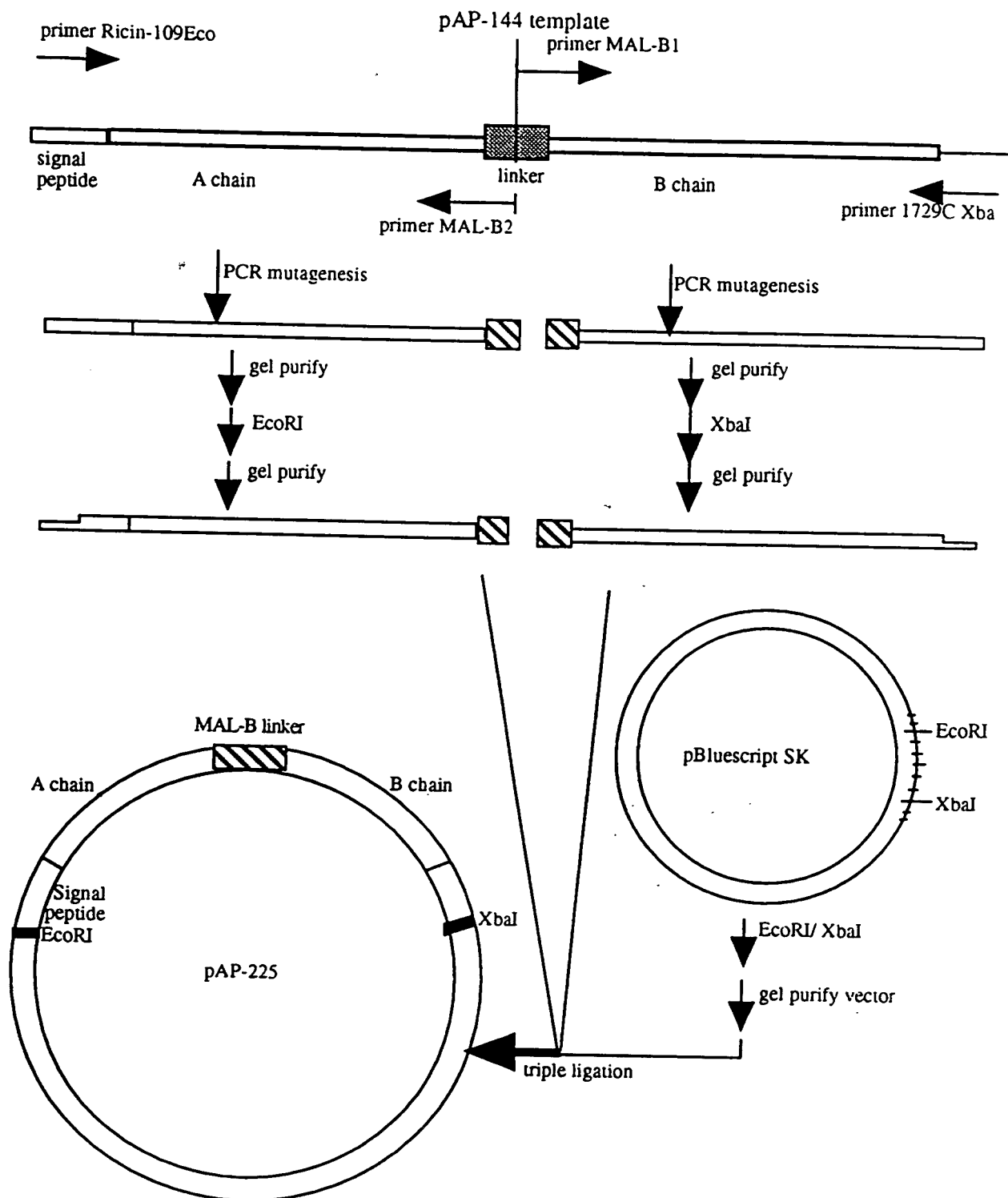
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

09551151-041400

37/254

FIGURE 8A



0955151-04-14-00

FIGURE 8B

WT preprorin linker

primer MAL-B1

5' - TCGGAGGACAATGATGAAGCTGATGTTGTATG -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTAAT

AGAAACGAAATATCCCGGTACACCAGGTTTAAATTA

3' - GGTAGCAGTGTCAAAAACGGCTAAAAGCCCTT -5'

primer MAL-B2

PCR mutagenesis

ligate with pBluescript SK

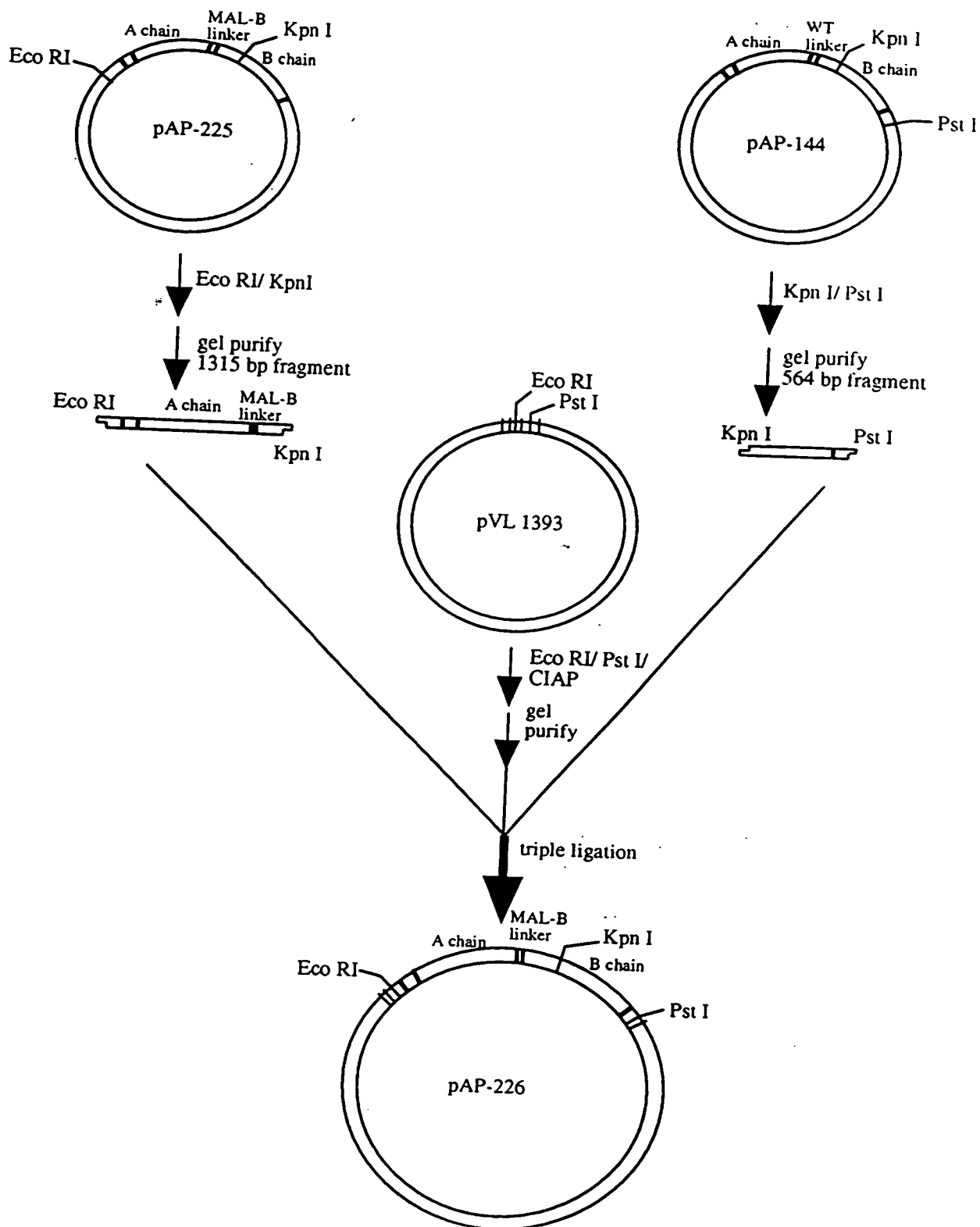
pAP 225 linker
(MAL-B variant)

TTGCCGATTTTCGGGGAATCGGAGGACAAATGATGAA

AACGGCTAAAAGCCCTTAGCCTCTGTTACTACTT

39/254

FIGURE 8C



09551151 04-14-00

40/254

FIGURE 8D

10 20 30 40 50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTGTTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTCCGGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCTTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCCGGCTACCGTGCTGGAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTTA
451 CGATATACATTCCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACCTGTTGAACG
501 TGGTAATCTGAGAGAAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAACT
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTTCCTTTATAATTTGCATCCAAATGATTTTCAAGAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTTCTCGGAAACGATCAGGTTA
801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTGGT
901 TCGTCACAGTTTTTGGCGATTTTCGGGGAATCGGAGGACAATGATGAAGC
AGCAGTGTCAAAAACGGCTAAAAGCCCCCTAGCCTCCTGTTACTACTTCG

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FIGURE 8D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAACCAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

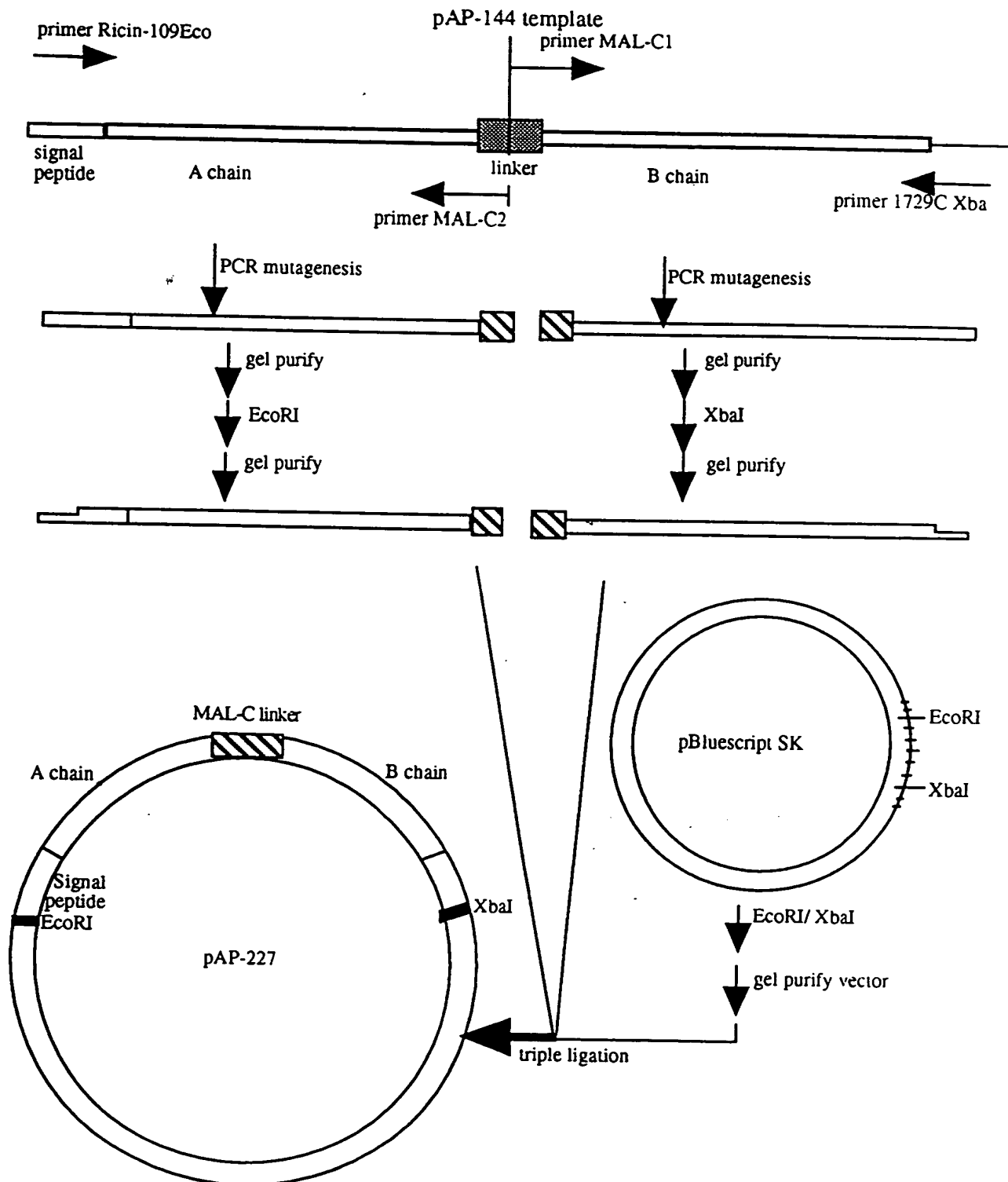
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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42/254

FIGURE 9A



00551151-04-1400

FIGURE 9B

WT preprorin linker

primer MAL-C1

5' - GCGATATCAGTTACTATGGCTGATGTTGTATG -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCGGTCACCCACGGTTTAAATTA

3' - GGTAGCAGTGTCAAAGTCCACCAATGTCCCTT -5'

primer MAL-C2

PCR mutagenesis

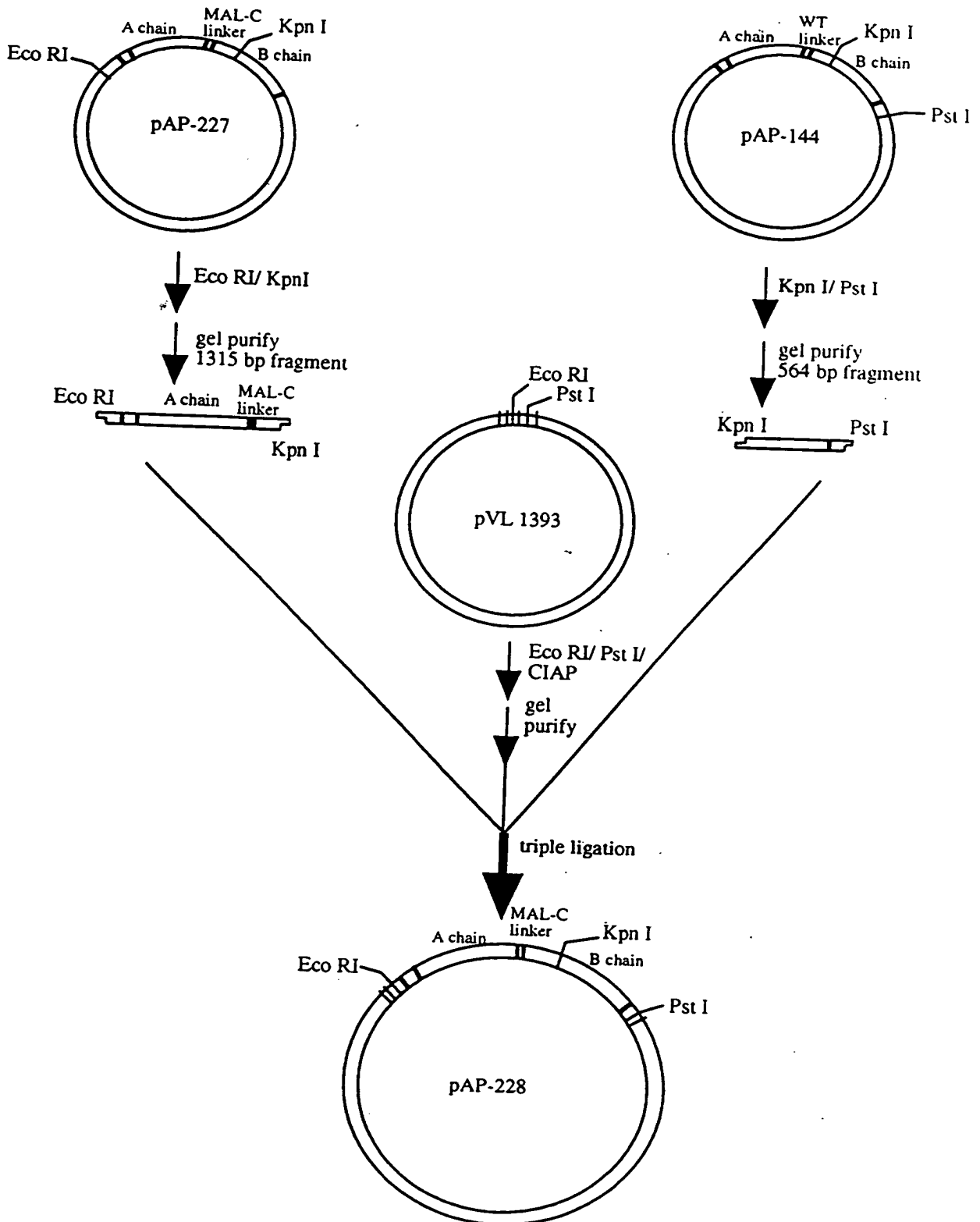
ligate with pBluescript SK

pAP 227 linker
(MAL-C variant)

CAGGTGTTACAGGGGAAGCATATCAGTTACTATG
GTCCACCAATGTCCCTTCGCTATAGTCAATGATAC

44/254

FIGURE 9C



09551151.04.14.00

45/254

FIGURE 9D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCTCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATTGGGTTAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTCATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTT

451 CGATATACATTTCGCTTTGGTGGTAATTATGATAGACTTGAACAATTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTTCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTTGGT

901 TCGTCACAGTTTTCAGGTGGTTACAGGGGAAGCGATATCAGTTACTATGGC
AGCAGTGTCAAAGTCCACCAATGTCCCCTTCGCTATAGTCAATGATACCG

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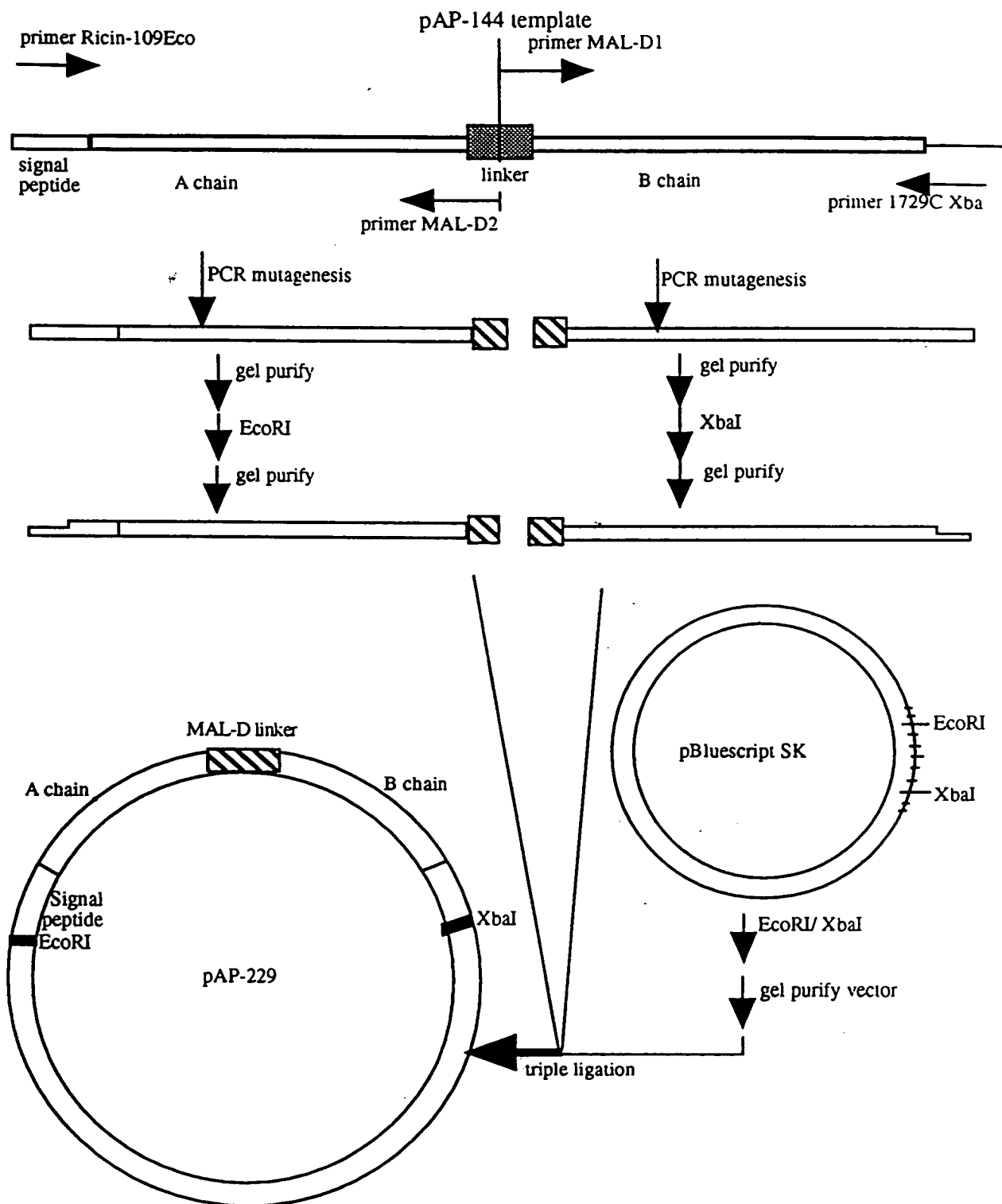
FIGURE 9D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCATCGTAGGTCGAAATG
 ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
 CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
 GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA
 1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTAACTACTTACG
 CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
 CCATGTCCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
 1201 ACTGATGCCACCCGCTGGCAAAATATGGGATAATGGAACCATCATAAATCC
 TGAATACGGTGGGCGACCGTTTATACCTATTACCTTGGTAGTATTTAGG
 1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
 GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG
 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
 AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
 1351 AATAATACACAACCTTTTGTACCAACCATTTGTTGGGCTATATGGTCTGTG
 TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
 GAACGTTTCGTTTATCACCTGTTTATACCTATCTCCTGACATCGTCACTTT
 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
 TCCGACTTGTGTGTCACCCGAGAAATACGTCCTACCAAGTTATGCAGGAGTC
 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
 GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
 1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA
 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
 AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA
 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCCTCTCCA
 CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
 1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
 ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA
 1751 CTCTTGACAGTGTGTGTGCTGCCATGAAAAATAGATGGCTTAAATAAAAA
 GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
 CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
 1851 TGCAG
 ACGTC

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47/254

FIGURE 10A



004740 "TST550

FIGURE 10B

WT prepronic linker

primer MAL-D1

5' - CTGTCGTTCCCTACTAATGCTGATGTTGT -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTAAT

AGAAACGAAAT*CCGGTCACCCACGGTTTAAATTA

3' - GGTAGCAGTGTCAAACGAAACCTCTCTTGCAAG -5'

primer MAL-D2

PCR mutagenesis

ligate with pBluescript SK

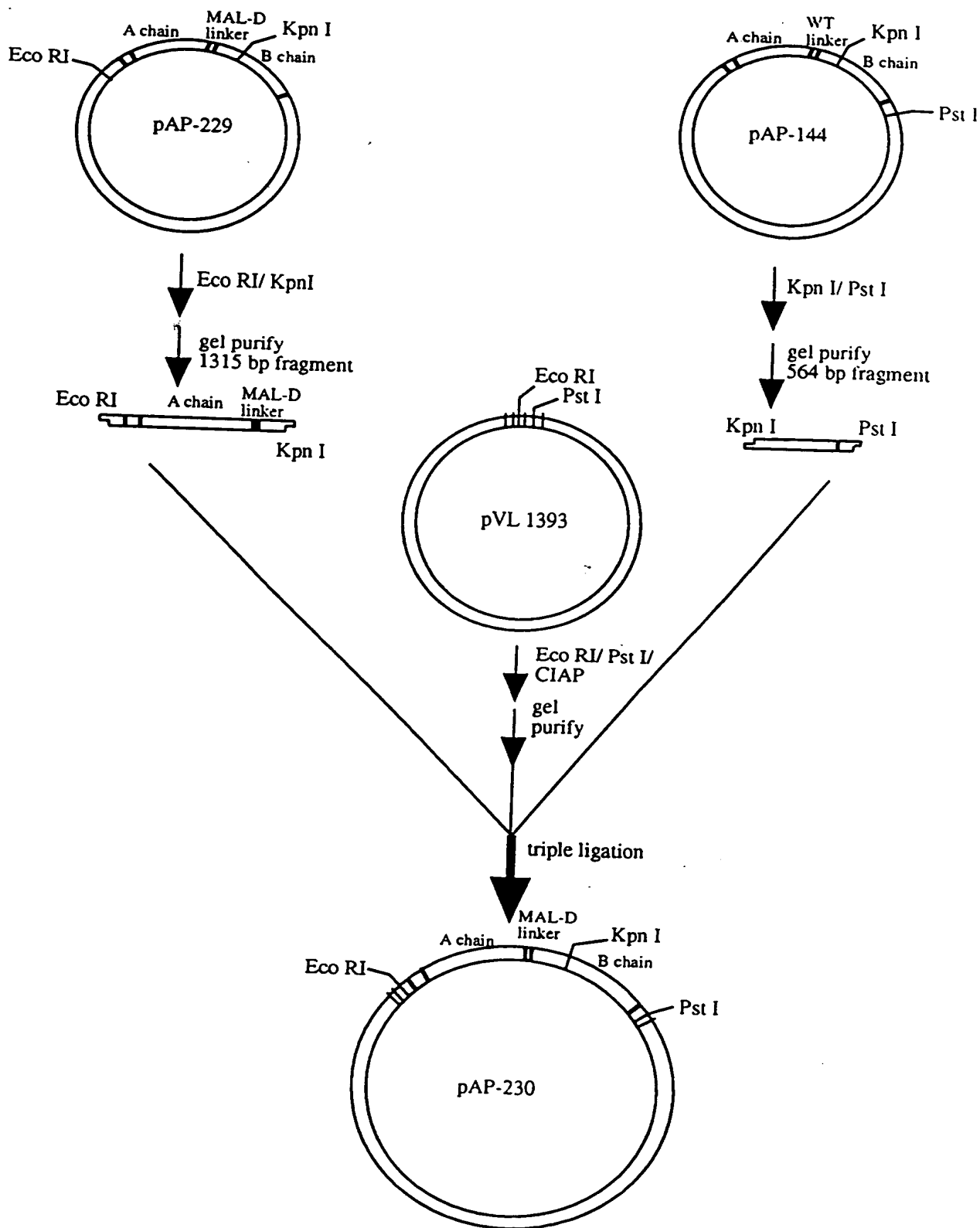
pAP 229 linker
(MAL-D variant)

GCTTTGGAGAGAACGTTCTGTCGTTCCCTACTAAT

CGAAACCTCTCTTGCAAGGACAGCAAGGGATGATTA

49/254

FIGURE 10C



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50/254

FIGURE 10D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACTGAGCTGATGTGAGACATGATATACCAGTGTGCGCAA
AGCAATTTGTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAAGTCAAGTTT

451 CGATATACATTTCGCTTTGGTGGTAATTATGATAGACTTGAACAACCTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTGTAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTGGT

901 TCGTCACAGTTTGCTTTGGAGAGAACGTTCCGTGTCGTTCCCTACTAATGC
AGCAGTGTCAAACGAAACCTCTCTTGCAAGGACAGCAAGGGATGATTACG

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51/254

FIGURE 10D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTACAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACCTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCCTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGTATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

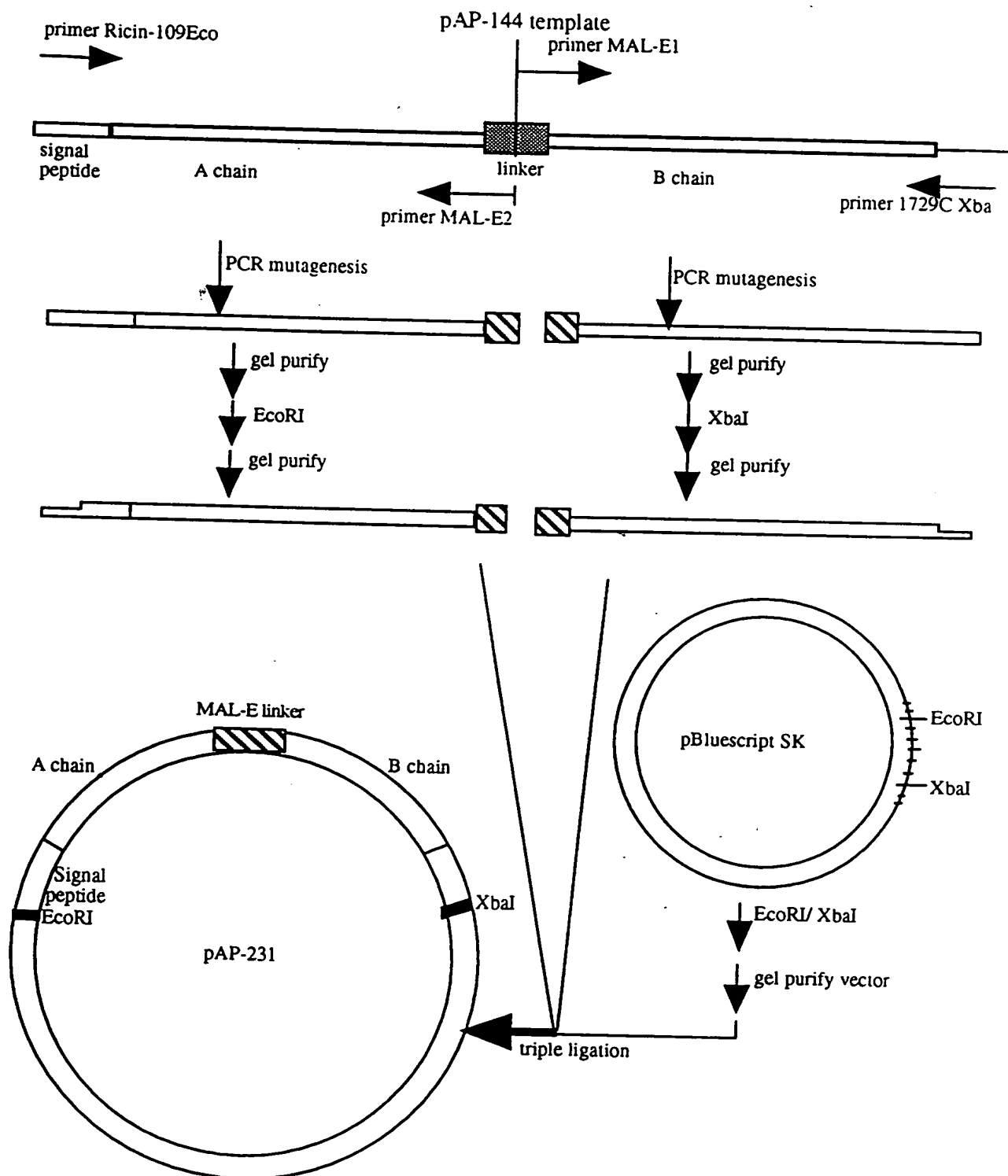
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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52/254

FIGURE 11A



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FIGURE 11B

WT preproricin linker

primer MAL-E1

5' - AATAATTCACAGCATCAGGCTGATGTTTGTATG -3'
 ***** * * * * *
 TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
 AGAAACGAAATATTCGGTCACCCACGGTTTAAATTA
 3' - GGTAGCAGTGTCAAATTTAAGGTTCTATACGAT -5'

primer MAL-E2

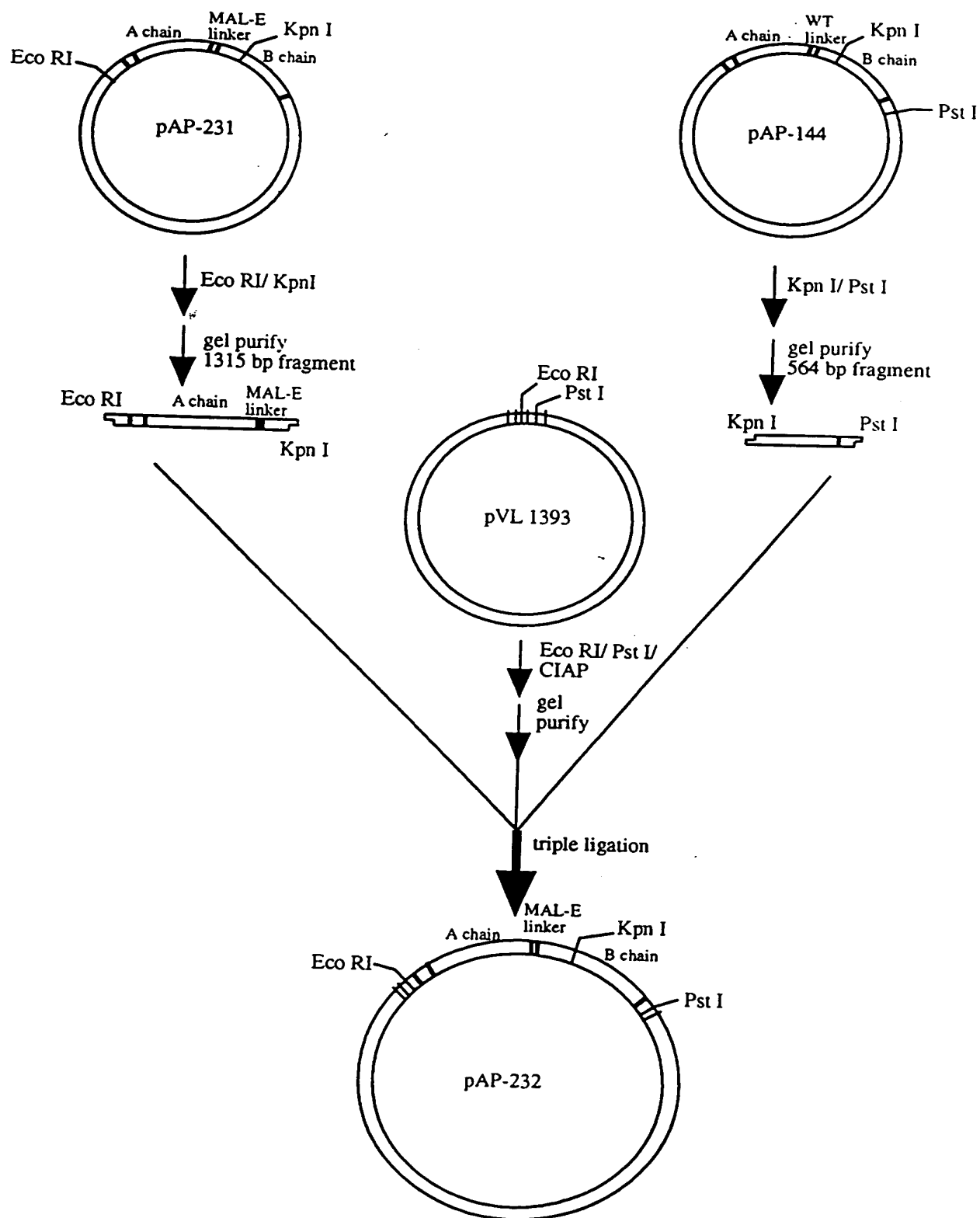
PCR mutagenesis
 ligate with pBluescript SK

pAP 231 linker
 (MAL-E variant)

AAATCCAAAGATATGCTAAATAATTCACAGCATCAG
 TTTAAGGTTCTATACGATTATTAAAGTGCCTAGTC

54/254

FIGURE 11C



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55/254

FIGURE 11D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAAGTACTACAAGTTT

451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTTGGT

901 TCGTCACAGTTTAAATTCCAAGATATGCTAAATAATTCACAGCATCAGGC
AGCAGTGTCAAATTTAAGGTTCTATACGATTTATTAAGTGTGCTAGTCCG

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FIGURE 11D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACCAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTCTGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCCTACTACCTTGGTAAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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57/254

FIGURE 12A

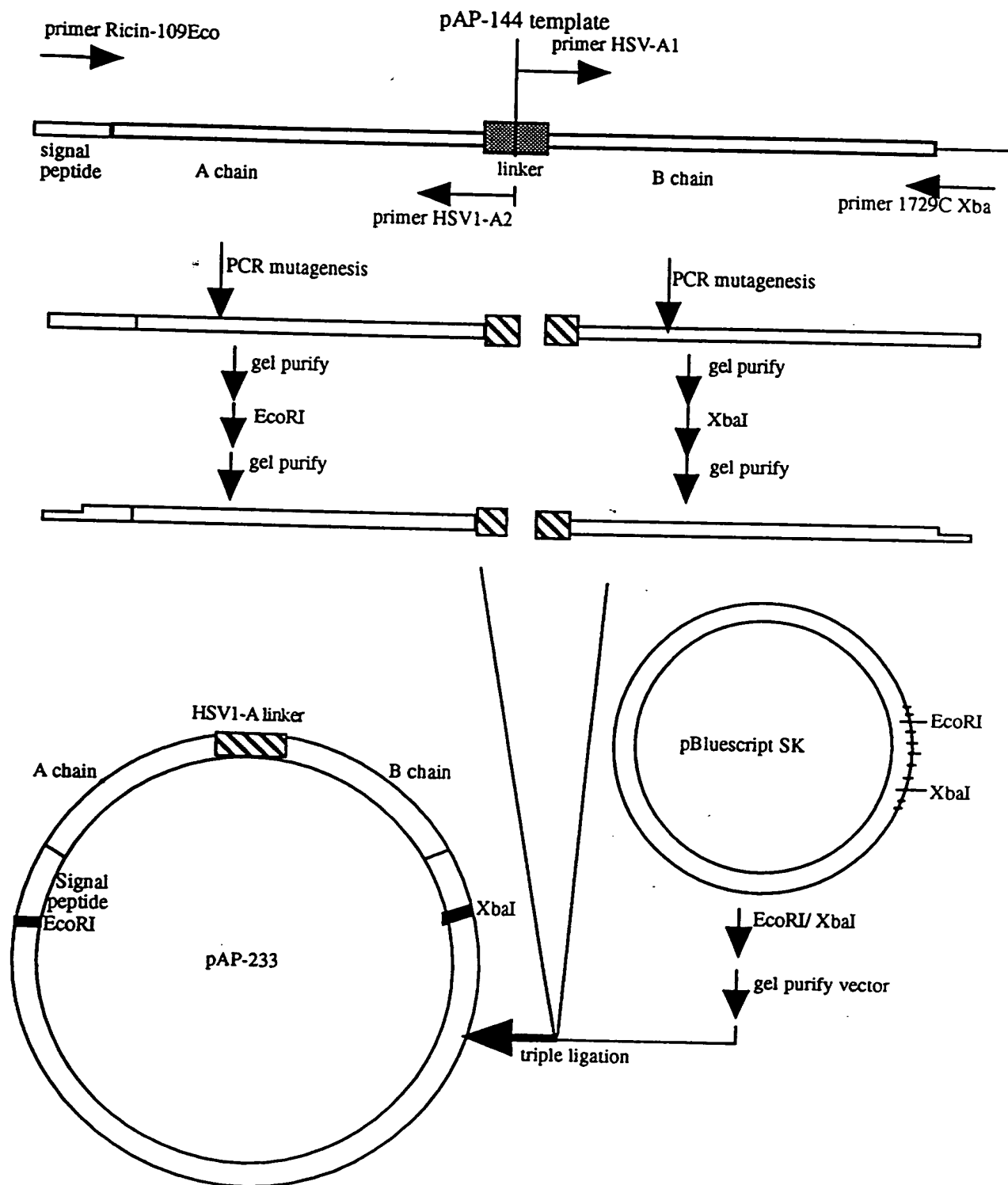


FIGURE 12B

WT preporicin linker

primer HSV1-A

5'- TCGTCGGCACATGTTAATGCTGATGTTTGT -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCGGT CACCACGGTTAAAAATTA

3' - AGCAGTGTCAAAAGACGCGAACATTTGCGT-5'

primer HSV1-A

PCR mutagenesis

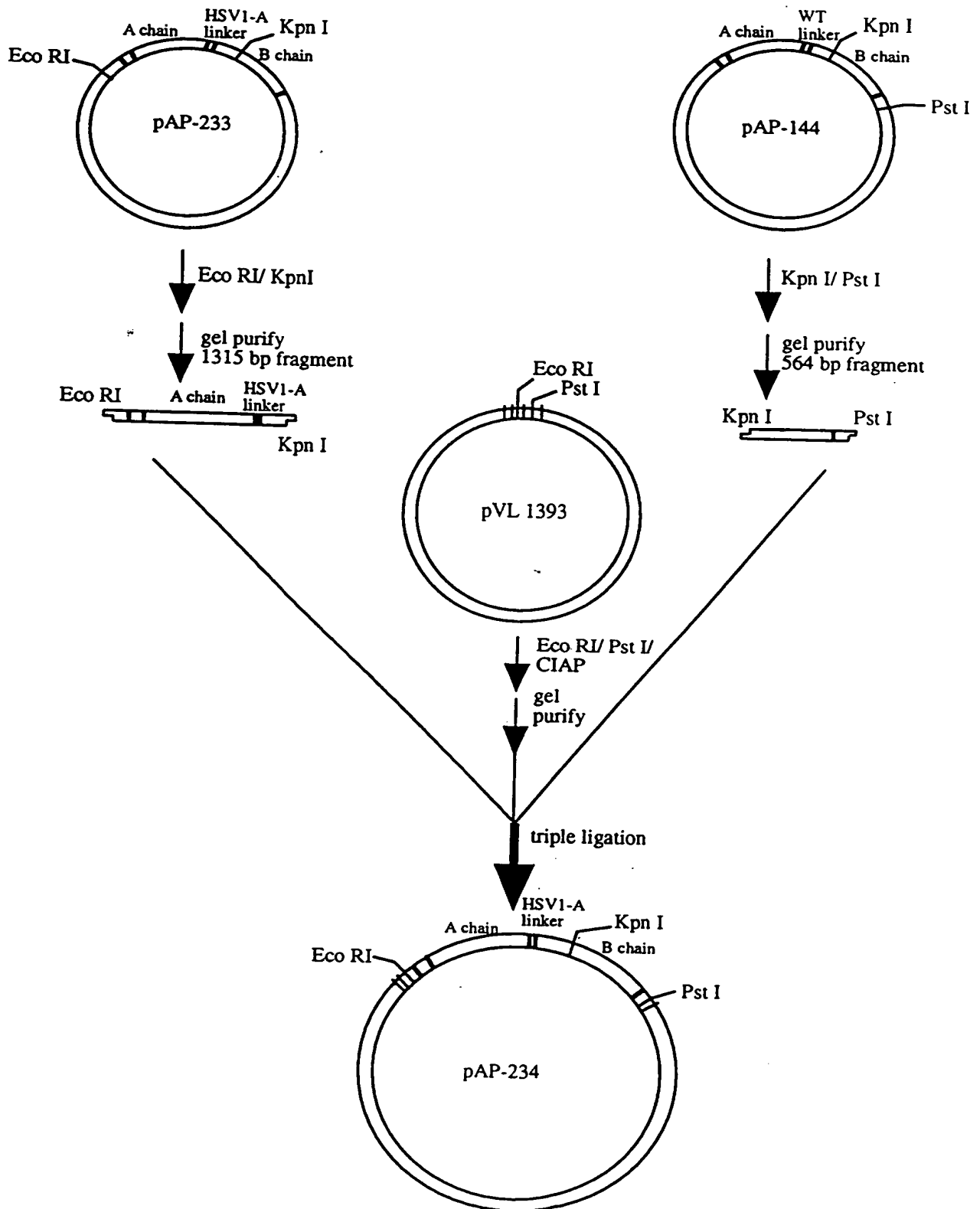
ligate with pBluescript SK

pAP 233 linker
(HSV1-A variant)

TCTGCGCTTGTAACGCATCGTCGGCACATGTTAAT
AGACGCGAACATTGCGTAGCAGCCGTGTACAATTA

59/254

FIGURE 12C



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60/254

FIGURE 12D

10 20 30 40 50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGAATC
101 AGGATAACAACATATTCGCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTCCGGG
CGCCACGGTGACACGTTTCGATGTGTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTTCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCTGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAATAAGTTTGA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCCTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCTGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCCTCGGAAACGATCAGGTTA
801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTGGT
901 TCGTCACAGTTTTCTGCGCTTGTAACGCATCGTCGGCACATGTTAATGC
AGCAGTGTCAAAGACGCGAACATTTGCGTAGCAGCCGTGTACAATTACG

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FIGURE 12D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCCTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

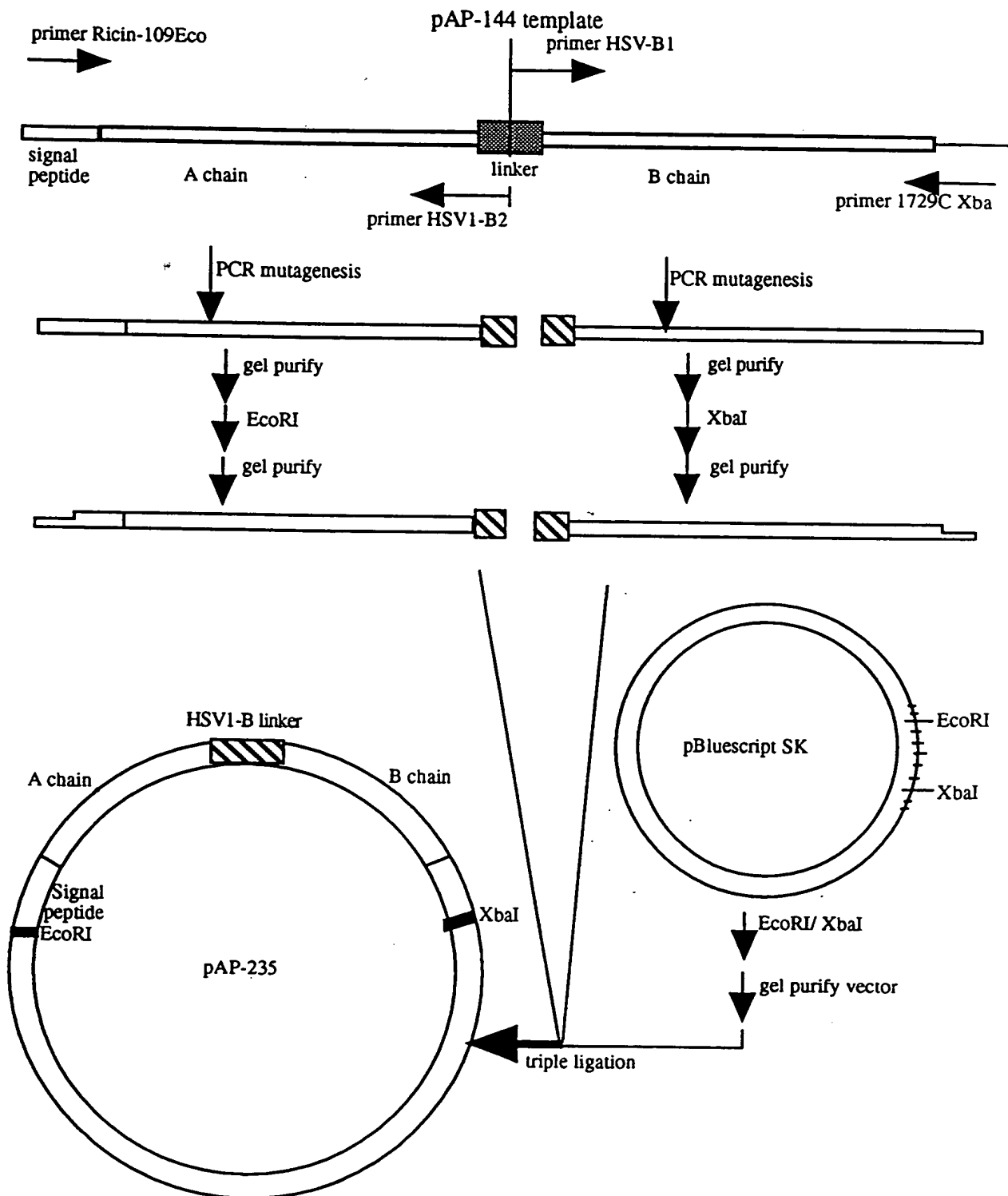
1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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62/254

FIGURE 13A



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FIGURE 13B

WT preprorin linker

primer HSV1-B

5' - TCGGAGAAATTTAAGAAATGCTGATGTTTGT -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCTGGTCAACCGGTTTAAATTA

3' - AGCAGTGTCAAAAGATGCATAAATGTCCGT-5'

primer HSV1-B

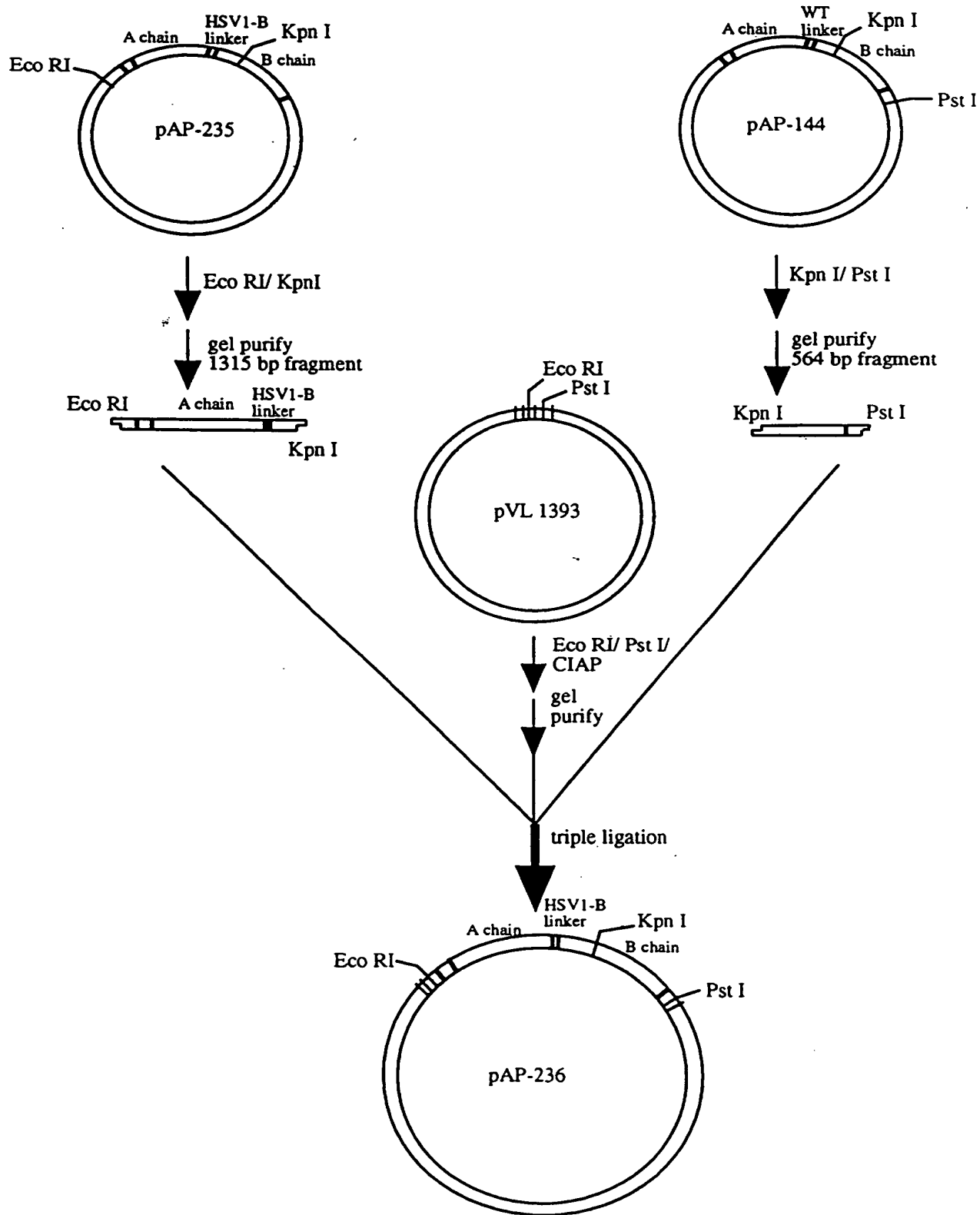
PCR mutagenesis
ligate with pBluescript SK

pAP 235 linker
(HSV1-B variant)

TCTACGTATTTACAGGCATCGGAGAAATTTAAGAAAT
AGATGCATAAATGTCCGTAGCCTCTTTAAATCTTA

64/254

FIGURE 13C



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65/254

FIGURE 13D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCTCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTCATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTTCATCCTGACA
ACACCAGCCGATGGCAGCACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAATAAGTTTGA

451 CGATATACATTTCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAT
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG
GACCGAGCAAGGAAATATTAACGTAGGTTTACTAAAGTCTTCGTCGTTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTCTCAGATTGGTTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTACGTATTTACAGGCATCGGAGAAATTTAAGAATGC
AGCAGTGTCAAAGATGCATAAATGTCCGTAGCCTCTTTAAATTCTTACG

09551.51.04.400

FIGURE 13D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGC GTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTT CAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGT TTA ACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTT CACAAATGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGT TACAACCATGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGAACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTT CATACTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTGCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGAATTGGTGT TAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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67/254

FIGURE 14A

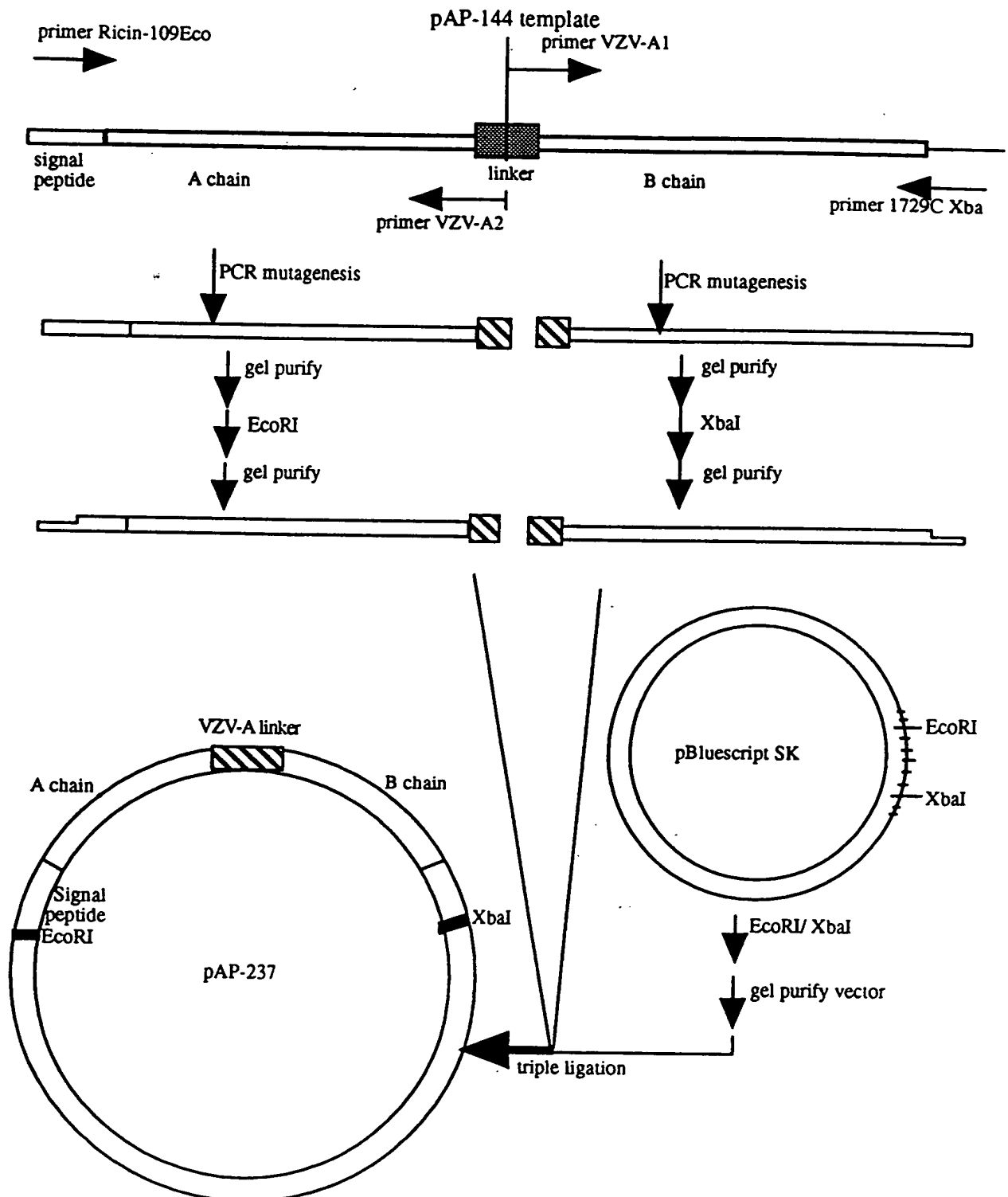


FIGURE 14B

WT preprorin linker

primer VZV-A1

5' - GTGGAGGCAAGTTCTAATGCTGATGTTTGT -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTAAT
AGAAACGAATATTCCGGT CACCACGGTTTAAATTA

3' - AGCAGTGTCAAAAGAGTCCTACATTTGCGT-5'

primer VZV-A2

PCR mutagenesis

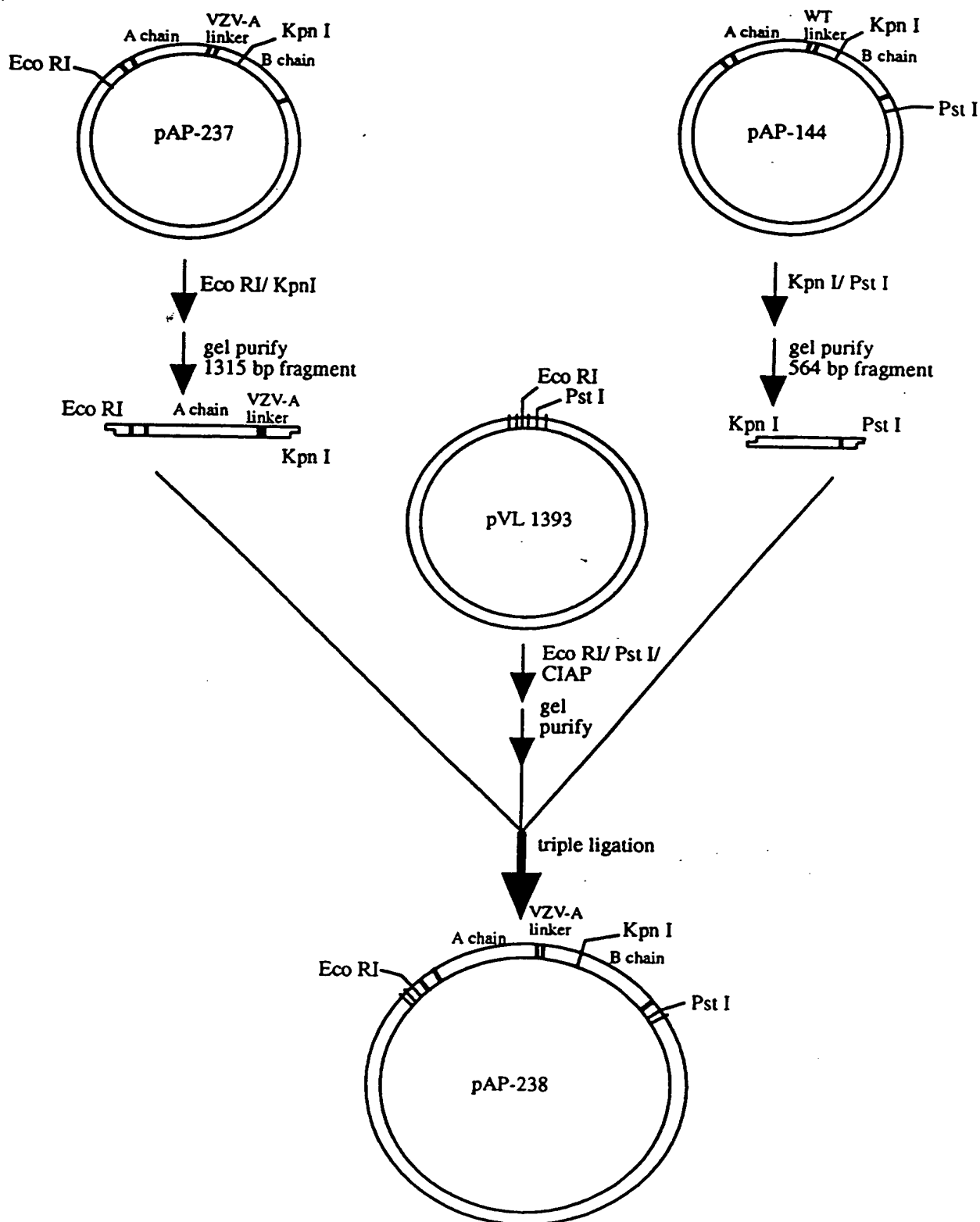
ligate with pBluescript SK

pAP 237 linker
(VZV-A variant)

TCTCAGGATGTAAACGACAGTGGAGGCAAGTTCTAAT
AGAGTCCCTACATTTGCGTCACCTCCGTTCAAGATTA

69/254

FIGURE 14C



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70/254

FIGURE 14D

	10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA				
51	GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTACATTAG				
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC				
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
	TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT				
151	GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTTCGCGG				
	CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC				
201	TCGTTTAAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA				
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251	ACAGAGTTGGTTTGCCTATAAAACCAACGGTTTATTTTAGTTGAACTCTCA				
	TGCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT				
301	AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351	TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTTCATCCTGACA				
	ACACCAGCCGATGGCAGCAGCTTTATCGCGTATAAAGAAAGTAGGACTGT				
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT				
	TAGTCCCTTCACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA				
451	CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC				
	GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG				
501	TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTCTTTTATAGCTCAACCCCTTACCAGGTGATCTCCTCC				
551	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601	CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG				
	GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT				
651	ATTCCAATATATTGAGGGAGAAATGCGCACGAGAAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT				
701	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA				
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTTATCAACCCCTCT				
751	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT				
	GAAAGGTGACGTTAAGTCTCAGATTGGTTCCCTCGGAAACGATCAGGTTA				
801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA				
	AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT				
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT				
901	TCGTACAGTTTTCTCAGGATGTAAACGCAGTGAGGCAAGTTCTAATGC				
	AGCAGTGTCAAAGAGTCTACATTTGCGTCACCTCCGTTCAAGATTACG				

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FIGURE 14D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTTACAAATGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTACGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAAATCGTCGCTGTAGTCCCTTGTACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTATACCTATCTCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGCTCTGCCATGAAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

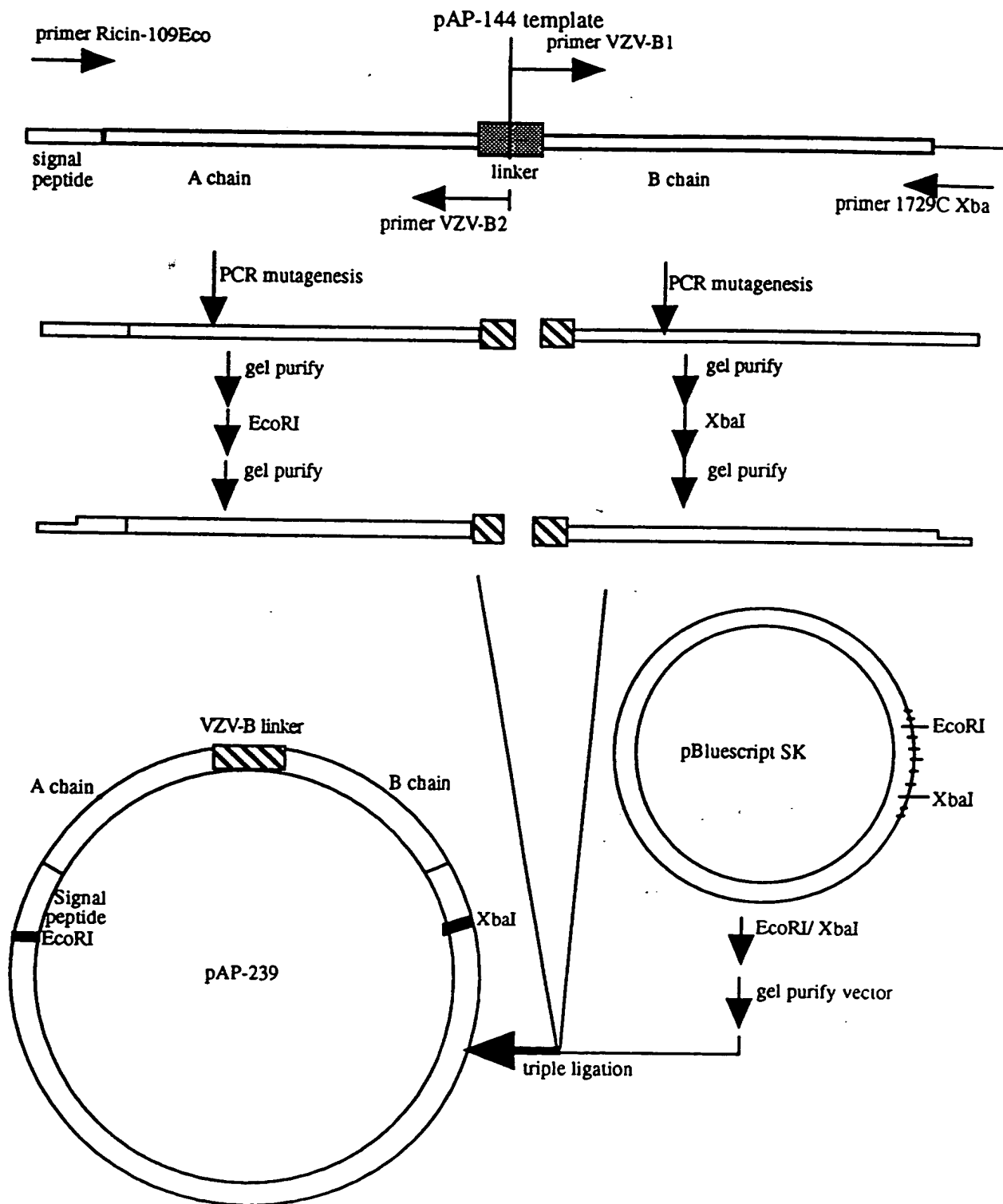
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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72/254

FIGURE 15A



0651151-041400

FIGURE 15B

WT preprorin linker

primer VZV-B1

5' - TCGACGGGATATGGTAATGCTGATGTTGT -3'

TCCTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCGGT CACCACGGTTTAAATTA

3' - AGCAGTGTCAAAAGACACATAAATGTCCGT-5'

primer VZV-A2

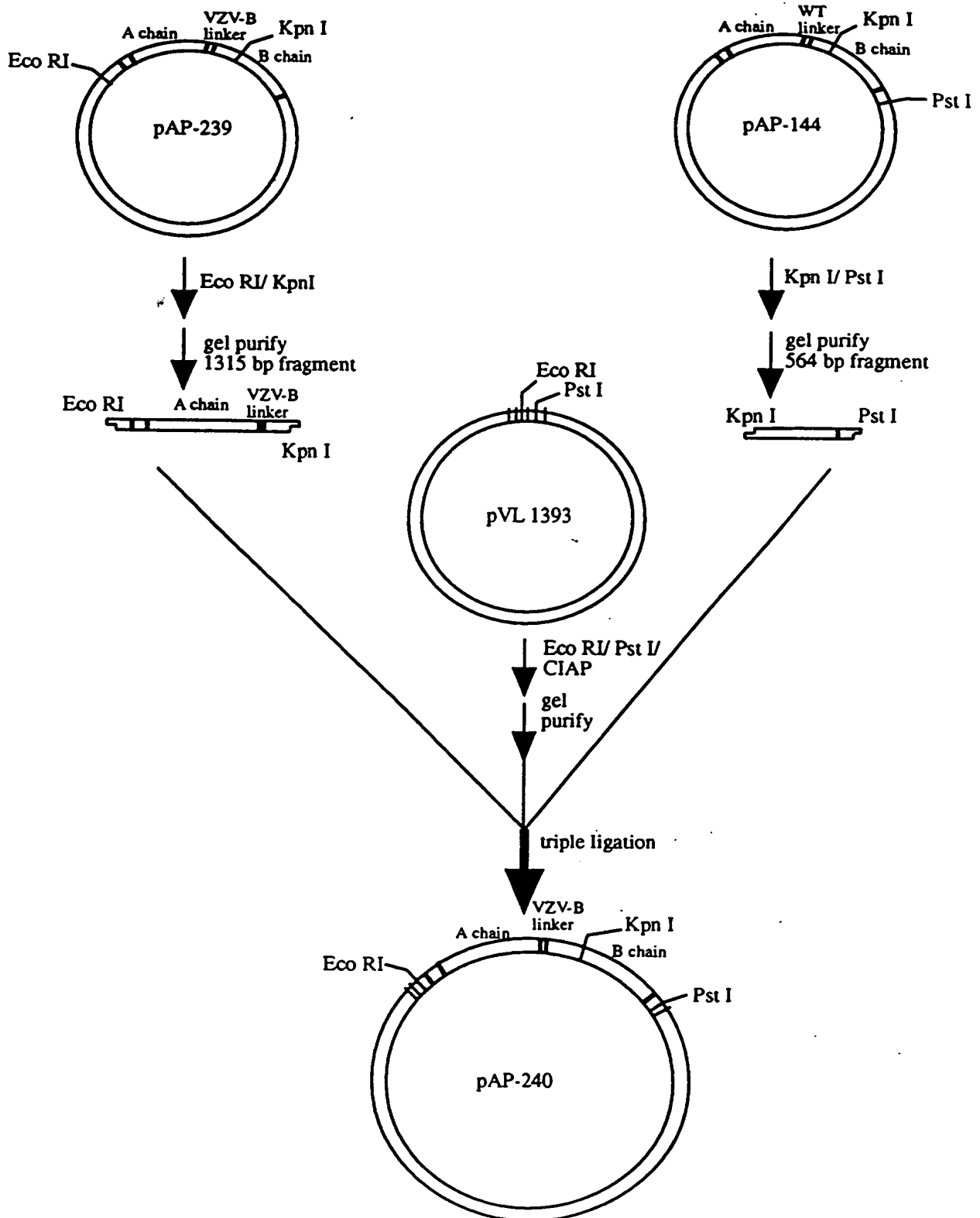
PCR mutagenesis
ligate with pBluescript SK

pAP 239 linker
(VZV-B variant)

TCTGTGTATTTACAGGCATCGACGGGATATGGTAAT
AGACACATAAATGTCCGTAGTGCCTTATACCATTA

74/254

FIGURE 15C



0951151-04400

75/254

FIGURE 15D

	10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA				
51	GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG				
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC				
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
	TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTGAAATGGTGT				
151	GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTGCGGG				
	CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC				
201	TCGTTTAAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCGCAA				
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251	ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA				
	TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT				
301	AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351	TGTGGTTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA				
	ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT				
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT				
	TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTAT				
451	CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC				
	GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTGTAACG				
501	TGGTAATCTGAGAGAAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC				
551	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601	CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG				
	GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCTGTT				
651	ATTCCAATATATTGAGGGAGAAAATGCGCACGAGAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT				
701	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA				
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT				
751	CTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT				
	GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA				
801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA				
	AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT				
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT				
901	TCGTACAGTTTTCTGTGTATTTACAGGCATCGACGGGATATGGTAATGC				
	AGCAGTGTCAAAGACACATAAATGTCCGTAGCTGCCCTATACCATTACG				

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76/254

FIGURE 15D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACACTACAATCCCTACCTTCTAAGGTGTTGCCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGAACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCTCAG
TCCGACTTGTTGTACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

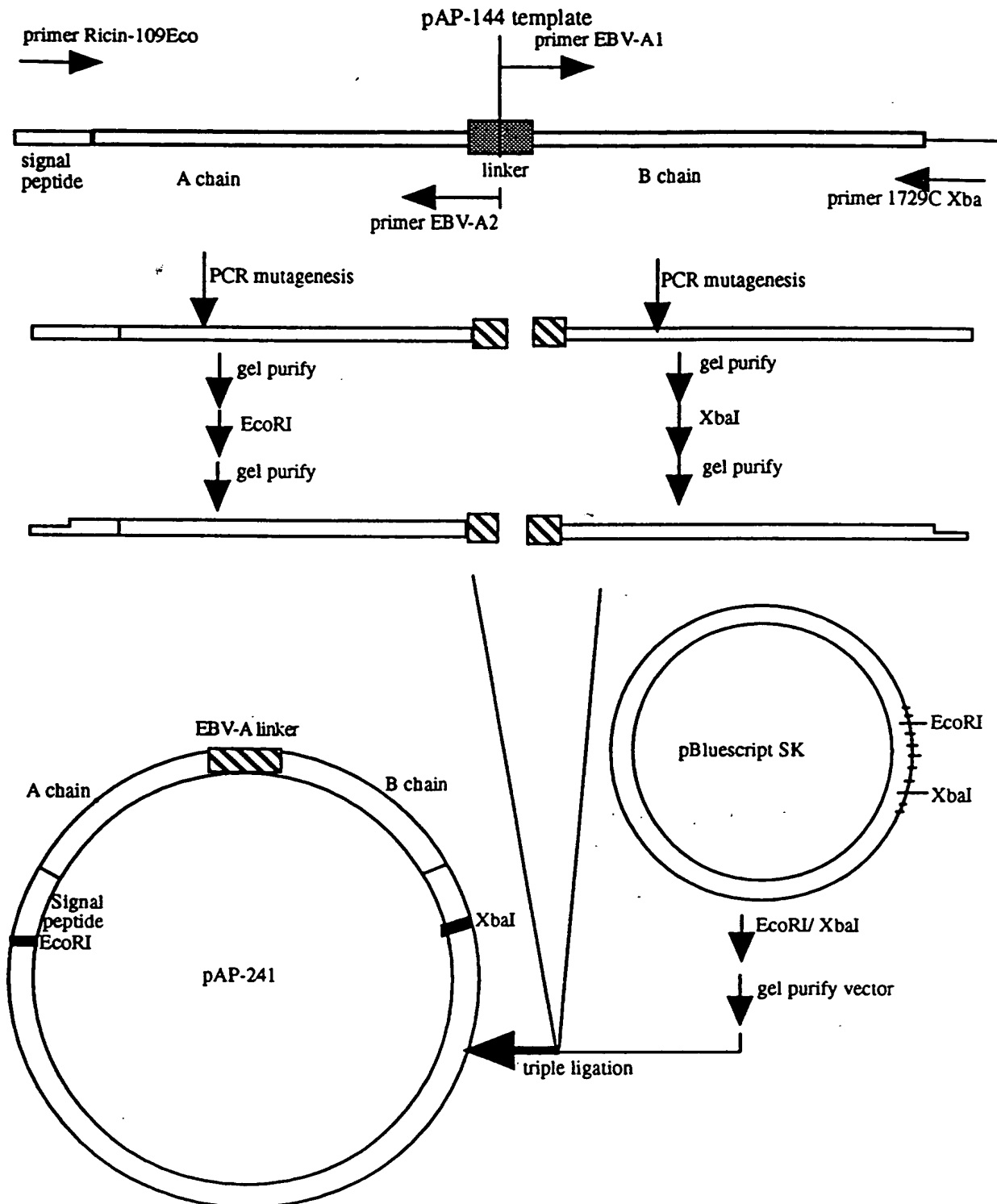
1851 TGCAG
ACGTC

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77/254

FIGURE 16A

PCR Mutagenesis of Preproricin Gene to Create an EBV-A Variant Gene a) Cloning Strategy



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FIGURE 16B

WT prepronicin linker

primer EBV-A1

5' - TCGGCGTCAGGTGTTAAATGCTGATGTTGT -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCCGTACCCACGGTTTAAATA

3' - AGCAGTGTCAAAAGATTTCGAACATGTCCGT-5'

primer EBV-A2

PCR mutagenesis

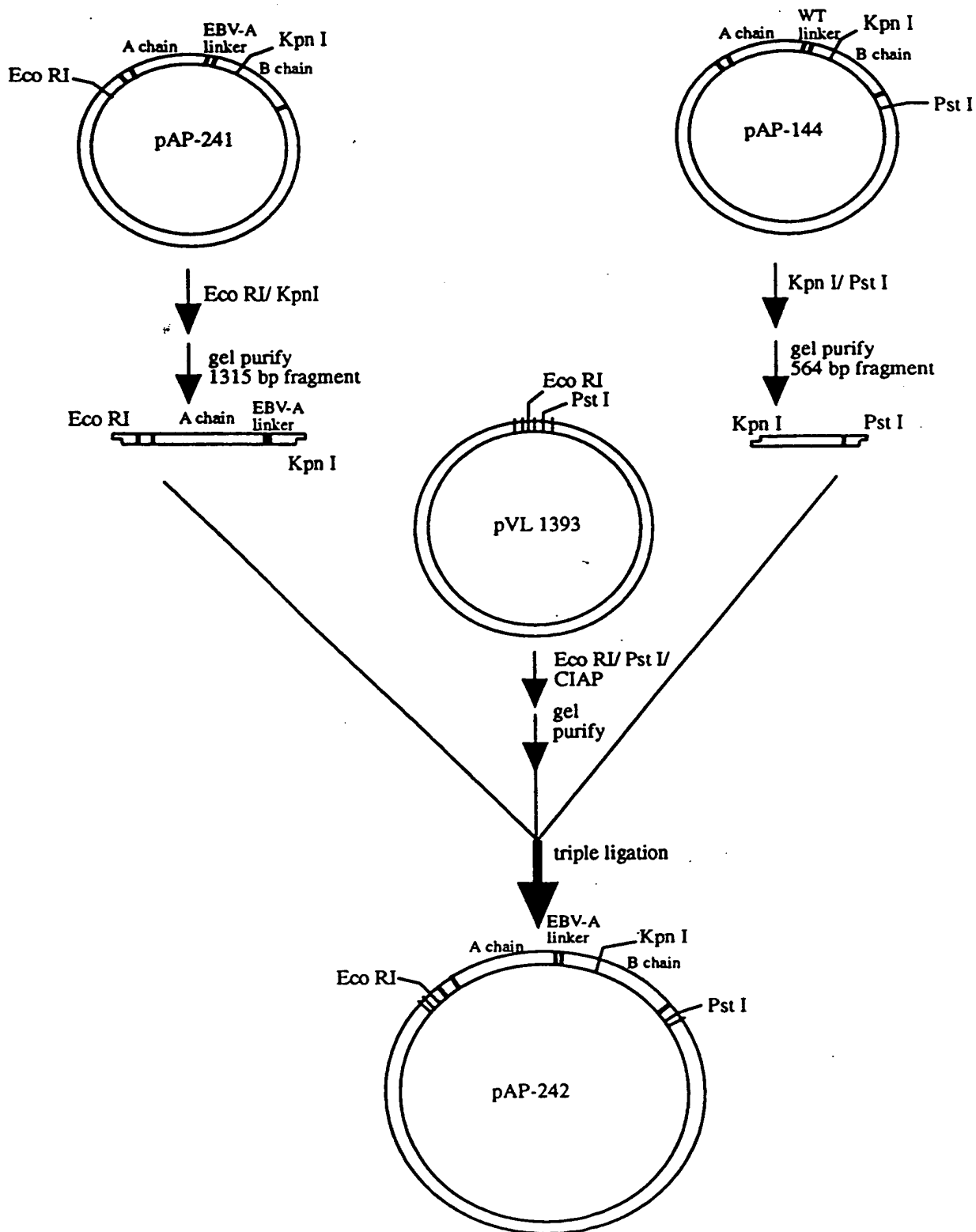
ligate with pBluescript SK

pAP241 linker
(EBV-A variant)

TCTAAGCTTGTCAGGCATCGGCGTCAGGTGTTAAAT
AGATTCGAACATGTCCGTAGCCGACGTCACAATTA

79/254

FIGURE 16C



065151-041400

80/254

FIGURE 16D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAAACACTGGAGCTGATGTGAGACATGATATACCAGTGTGCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA

451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAACTACTATCTGAACTTGTGTAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAT
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTTCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG
GACCGAGCAAGGAAATATTAACGTTAGGTTTACTAAAGTCTTCGTCGTTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTCTAAGCTTGTACAGGCATCGGCGTCAGGTGTTAATGC
AGCAGTGTCAAAAGATTGGAACATGTCCGTAGCCGCAGTCCACAATTACG

095151.04.400

FIGURE 16D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGC GTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCTAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

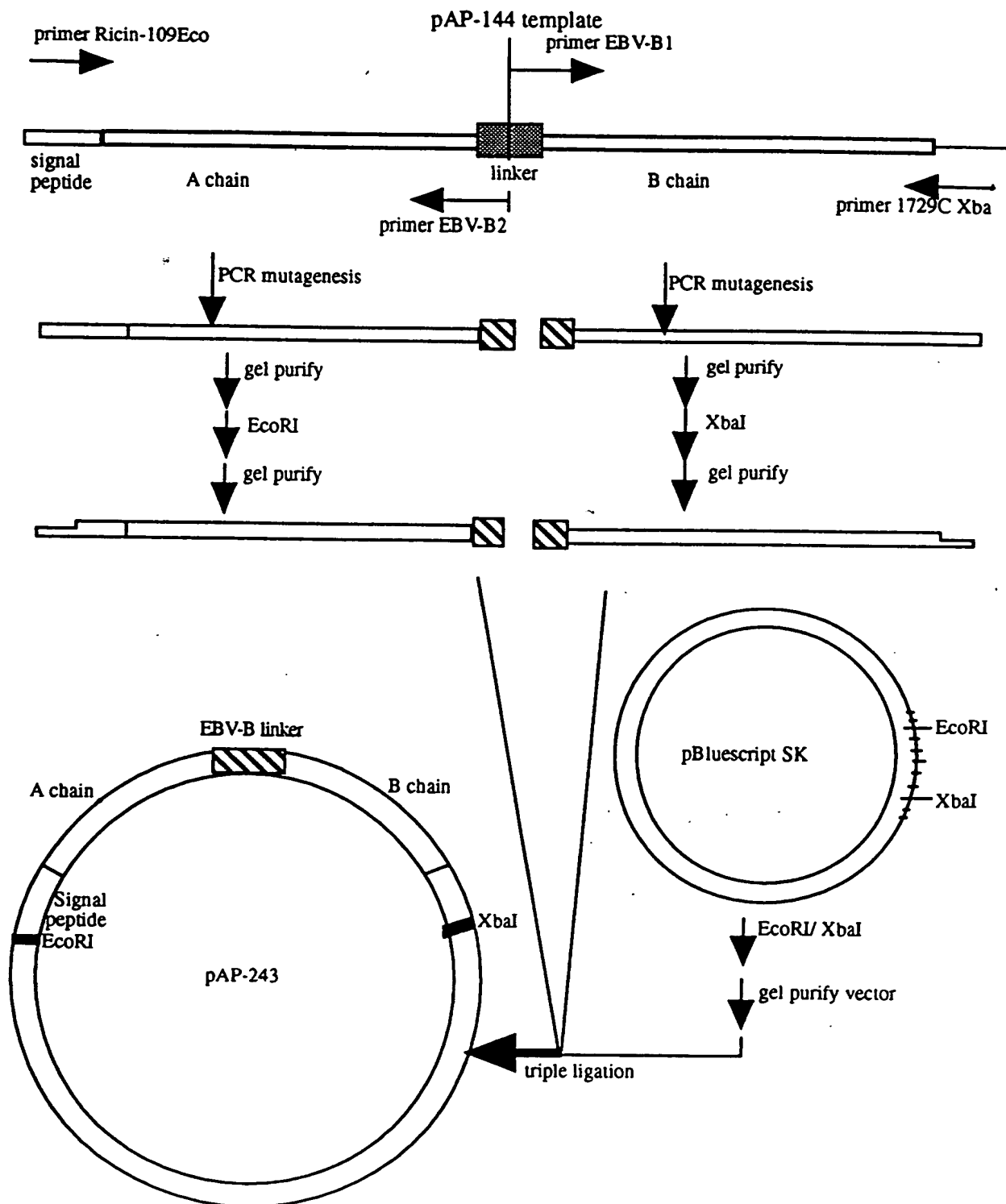
1751 CTCTTGCAGTGTGTGTCTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

82/254

FIGURE 17A



00440"TSF560

FIGURE 17B

WT preprorocin linker

primer EBV-B1

5' - TCGGACGCACTGATAATGCTGATGTTGT - 3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCGGT*
CACCACGGTTTAAATTA

3' - AGCAGTGTCAAAAAGACATAGATTTCCT-5'

primer EBV-B2

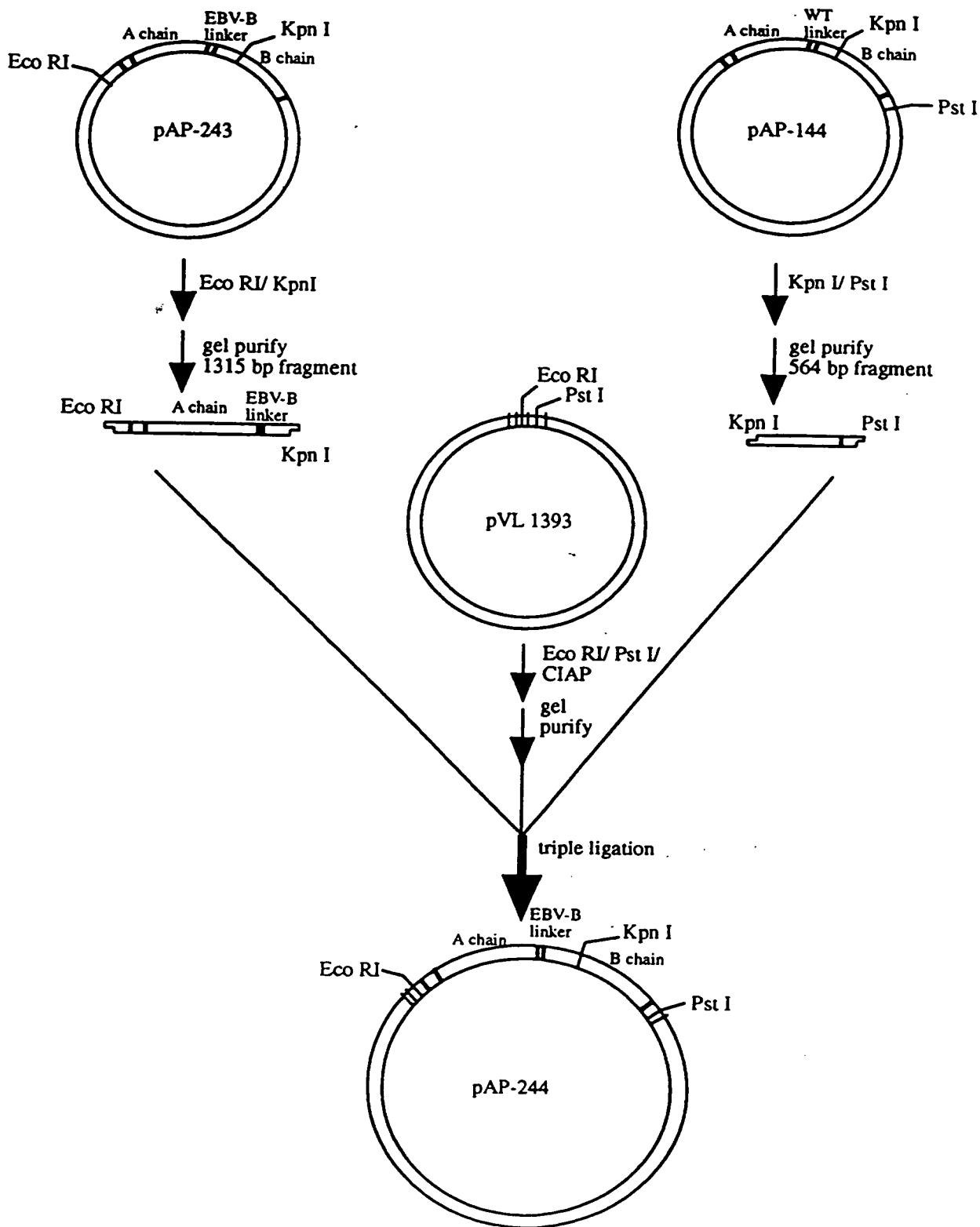
PCR mutagenesis
ligate with pBluescript SK

pAP 243 linker
(EBV-B variant)

TCTTCGTATCTAAAGGCATCGGACGCCCTGATAAT
AGAAGCATAGATTTCCTGTAGCCTGCCGTGGACTATTA

84/254

FIGURE 17C



00440" TST5560

85/254

FIGURE 17D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA

451 CGATATACATTTCGCCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAACGCTAGGTTTACTAAAGTCTTCGTCGTTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTTGGT

901 TCGTCACAGTTTTCTTCGTATCTAAAGGCATCGGACGCACCTGATAATGC
AGCAGTGTCAAAGAAGCATAGATTTCCGTAGCCTGCGTGGAATATTACG

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FIGURE 17D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGC GTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTT CAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGT TTA ACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAA AATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGT TACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGA AACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTT CATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGT TAGAT
AGTTC TTACTACCTTGGTAAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

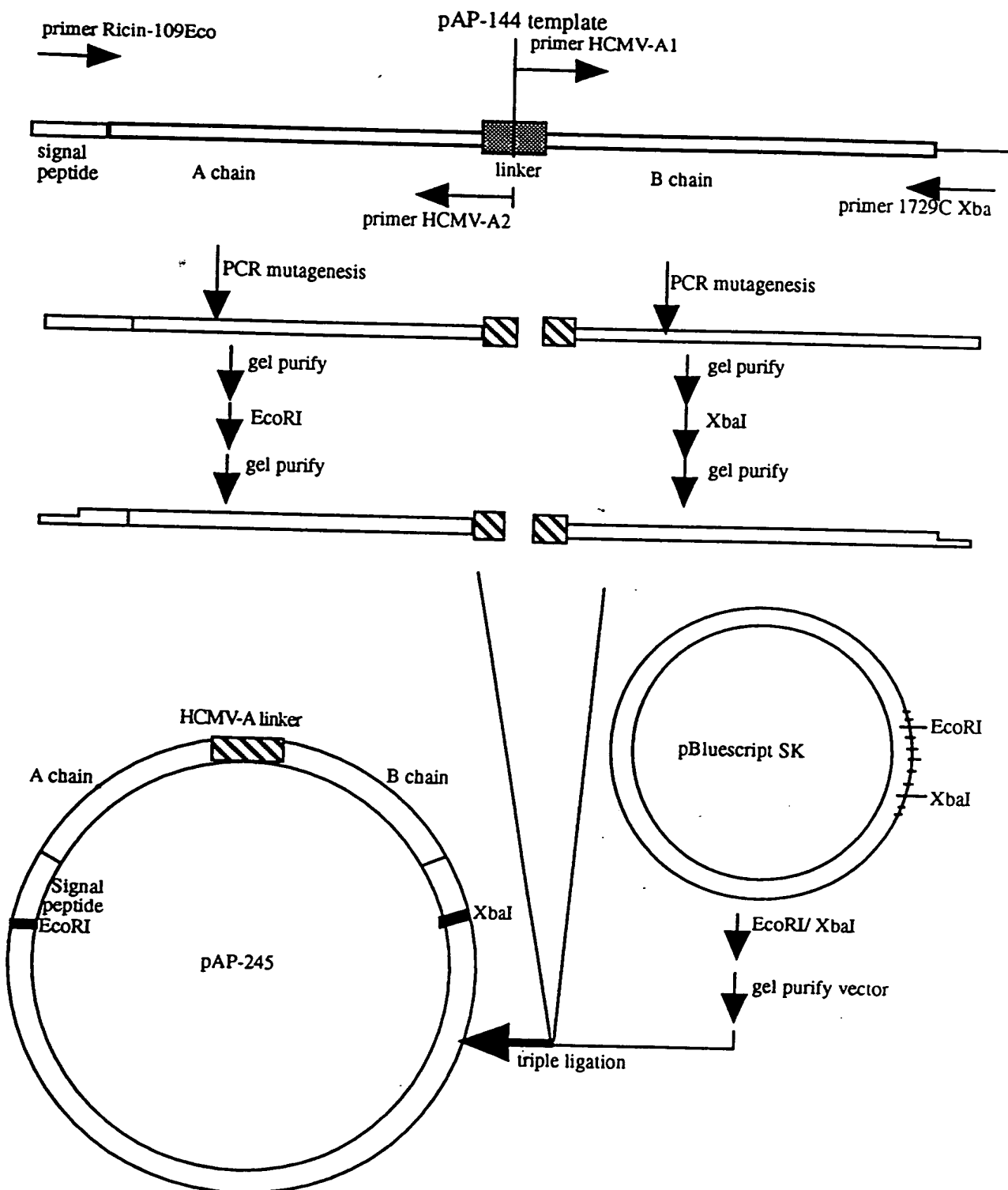
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

004F40" TSTT560

87/254

FIGURE 18A



004740 "T551560

FIGURE 18B

WT preprorcin linker

primer HCMV-A1

5' - TCGTGTAGACTTGCCTAATGCTGATGTTGT -3'
 *** **
 TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
 AGAAACGAATATTCCGGT CACCACGGTTAAATTA
 **
 3' - AGCAGTGTCAAAAAGACCCCAACATTTACGT-5'

primer HCMV-A2

PCR mutagenesis

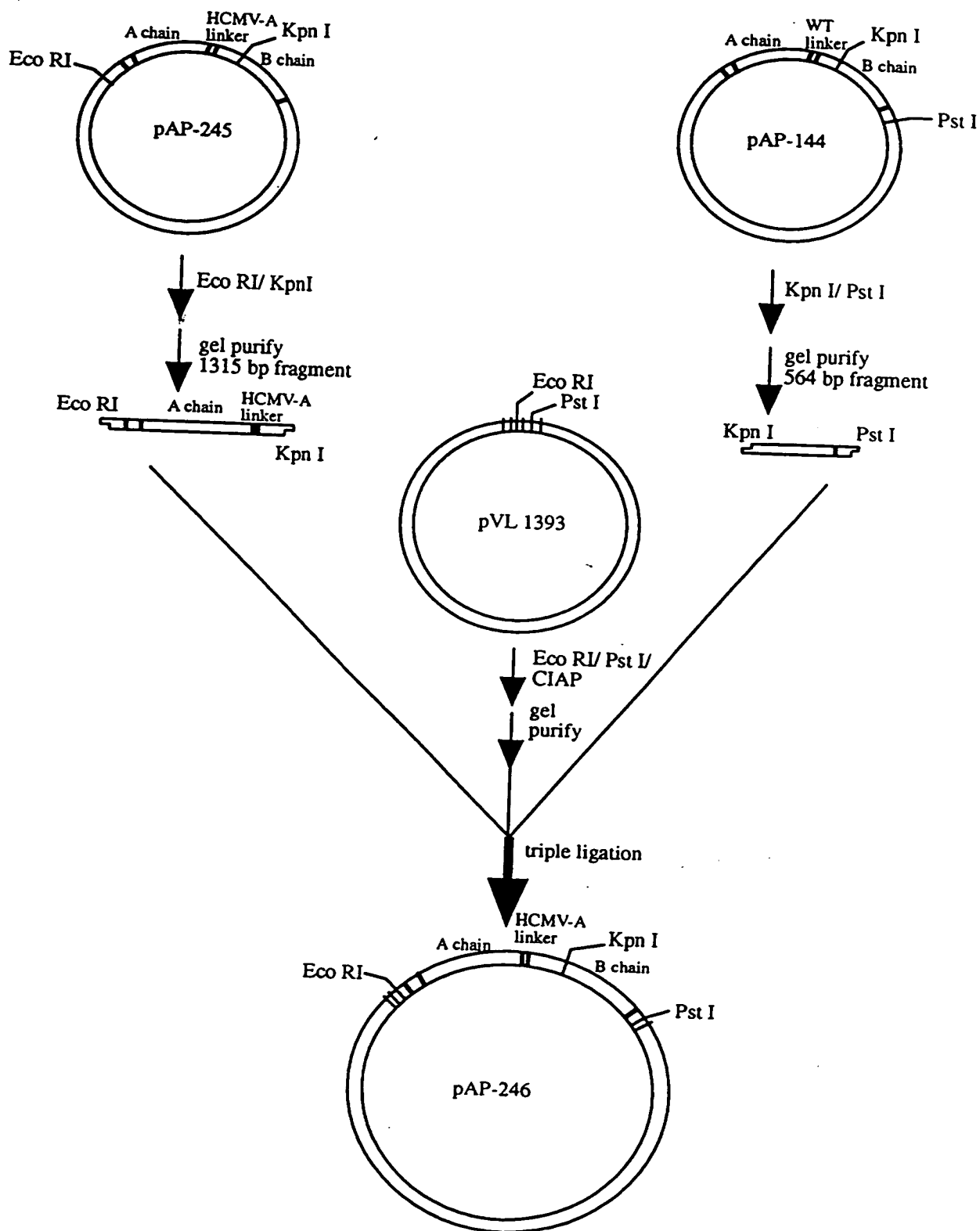
ligate with pBluescript SK

pAP 245 linker
 (HCMV-A variant)

TCTGGGGTTGTAAATGCATCGTGTAGACTTGCTAAT
 AGACCCCAACATTTACGTAGCACATCTGAACGATTA

89/254

FIGURE 18C



004400" FSTT560

90/254

FIGURE 18D

10 20 30 40 50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACTGAGCTGATGTGAGACATGATATACCAGTGTGCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCTTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTGTTGCGCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCTGACA
ACACCAGCCGATGGCAGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAACAAGTTTAA
451 CGATATACATTTCGCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG
501 TGGTAATCTGAGAGAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCTCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTTGGT
901 TCGTCACAGTTTTCTGGGGTGTAAATGCATCGTGTAGACTTGCTAATGC
AGCAGTGTCAAAAGACCCCAACATTTACGTAGCACATCTGAACGATTACG

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91/254

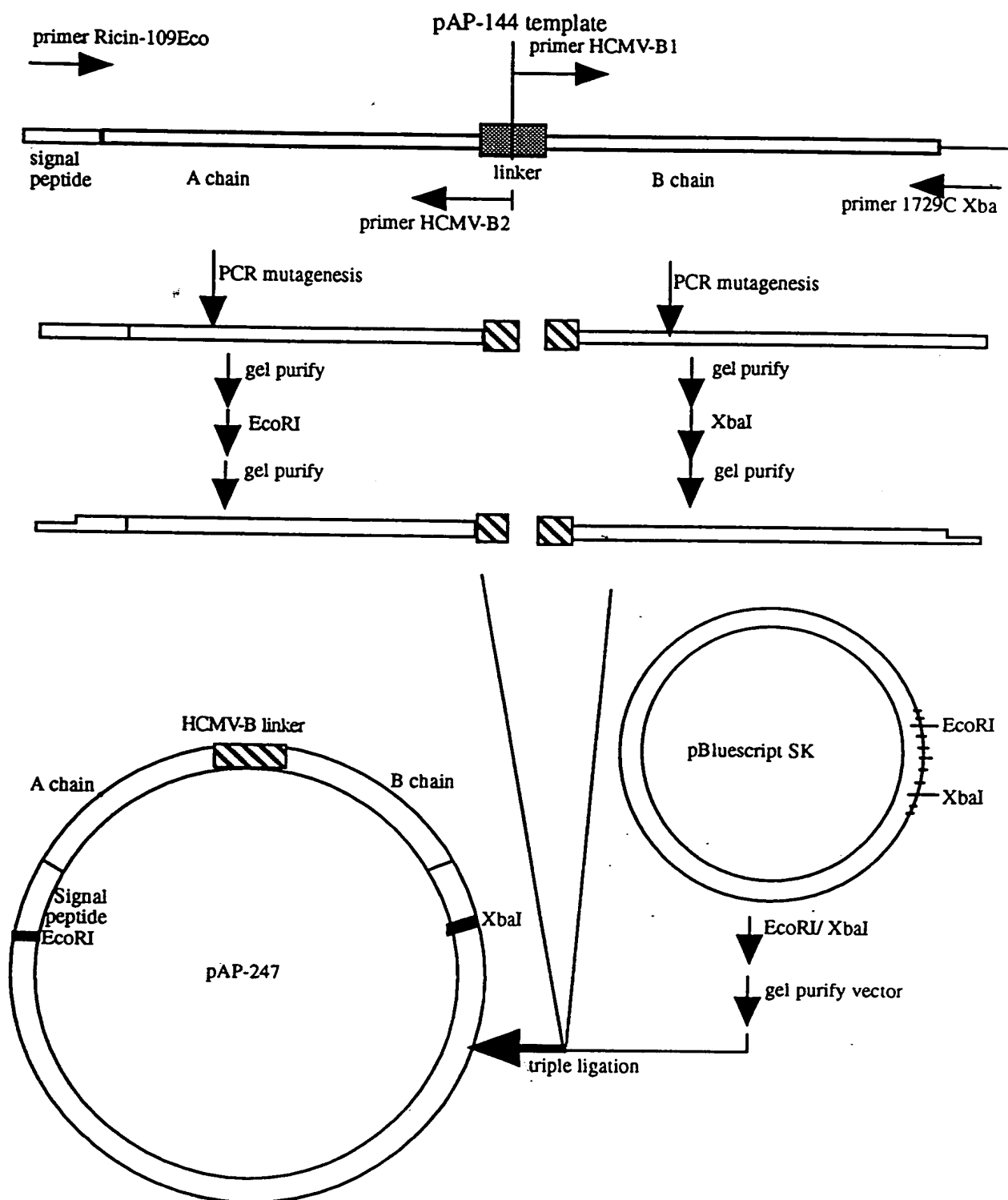
FIGURE 18D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG
1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351 AATAATACACAACCTTTTGTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAACAATGTTGGTAACAACCCGATATACCAGACAC
1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTATACCTATCTCCTGACATCGTCACTTT
1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA
1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCAATCTA
1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA
1751 CTCTTGCAGTGTGTGTCTCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851 TGCAG
ACGTC

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92/254

FIGURE 19A



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FIGURE 19B

WT preprorin linker

primer HCMV-B1

5' - TCGGTGTCACCTGAAATGCTGATGTTGT -3'

TCTTTCGTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCGGTCACCCACGGTTTAAATTA

3' - AGCAGTGTCAAAAAGACATACATTTCCCGT-5'

primer HCMV-B2

PCR mutagenesis

ligate with pBluescript SK

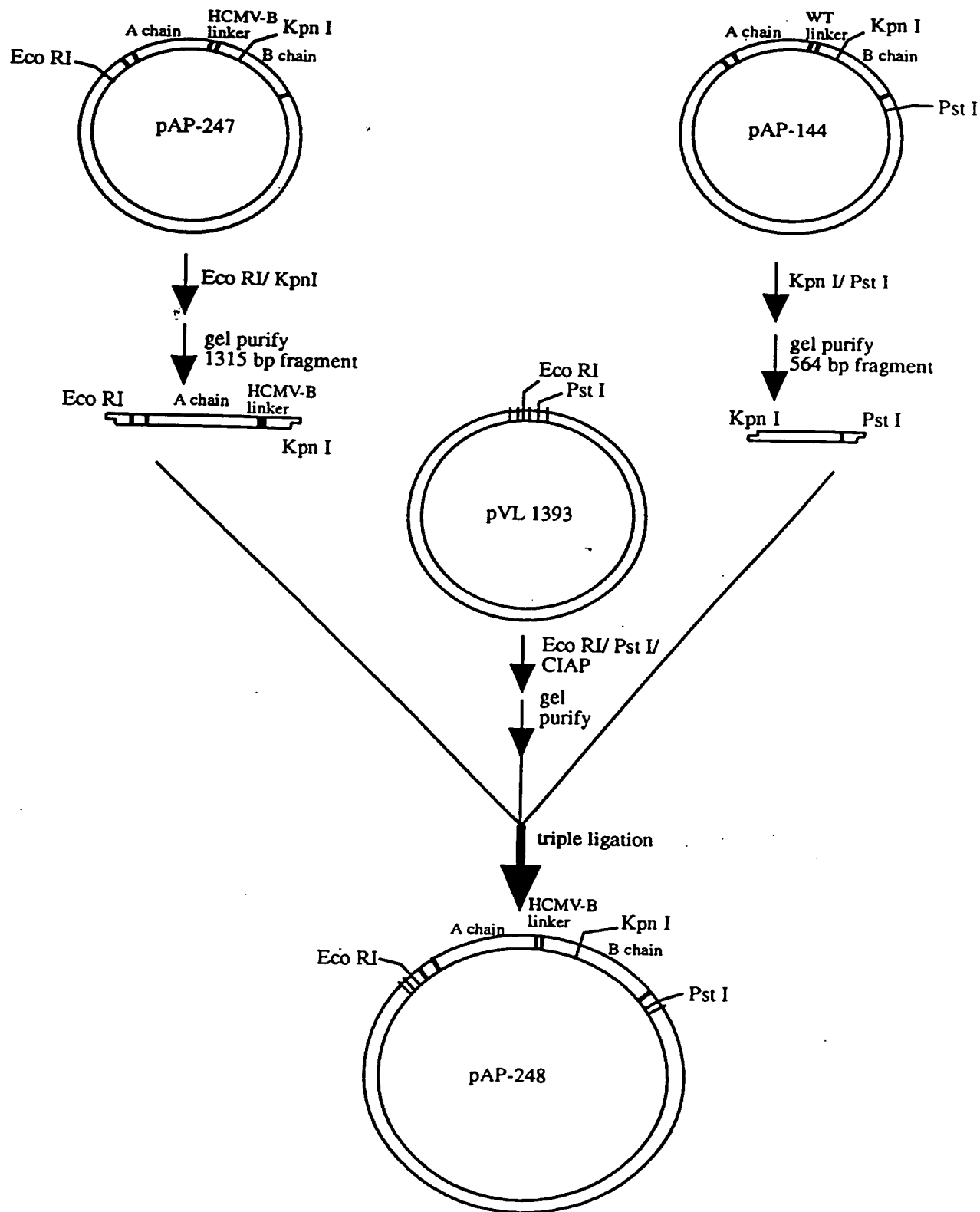
pAP 247 linker

(HCMV-B variant)

TCTTCGTATGTAAAGGCATCGGTGTCACCTGAAAT
AGAAGCATACATTTCCGGTAGCCACAGTGGACTTTTA

94/254

FIGURE 19C



004740" TST560

95/254

FIGURE 19D

	10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA				
51	GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG				
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGAATC				
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
	TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT				
151	GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG				
	CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC				
201	TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA				
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251	ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA				
	TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTGAGAGT				
301	AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351	TGTGGTTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA				
	ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT				
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT				
	TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAATAAAGTTTGA				
451	CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC				
	GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACCTGTTGAACG				
501	TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC				
551	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601	CTGGCTCGTTCCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG				
	GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT				
651	ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT				
701	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA				
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTTATCAACCCCCCTCT				
751	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT				
	GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCCTCGGAAACGATCAGGTTA				
801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA				
	AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT				
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT				
901	TCGTACAGTTTTCTTCGTATGTAAAGGCATCGGTGTACCTGAAAATGC				
	AGCAGTGTCAAAAGAAGCATACATTTCCGTAGCCACAGTGGACTTTTACG				

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FIGURE 19D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCCTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCTTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTTGCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAATATGGTTACCATTATTTTGTATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACAGGACGGTACTTTTATCTACCGAATTTATTTTTT

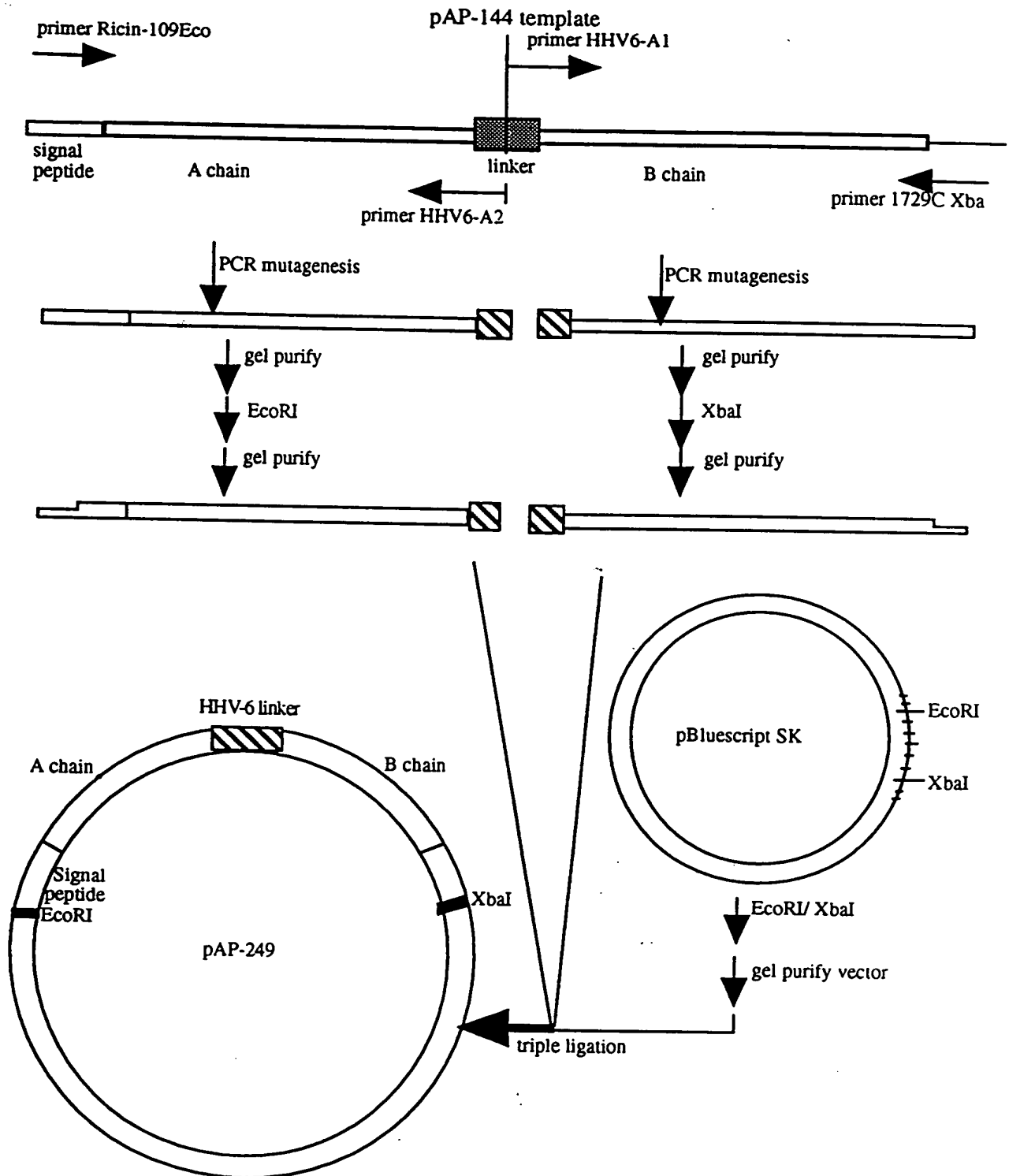
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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97/254

FIGURE 20A



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FIGURE 20B

WT preproricin linker

primer HHV6-A1

5' - TCGGTGCCAAATTTAAT -3'

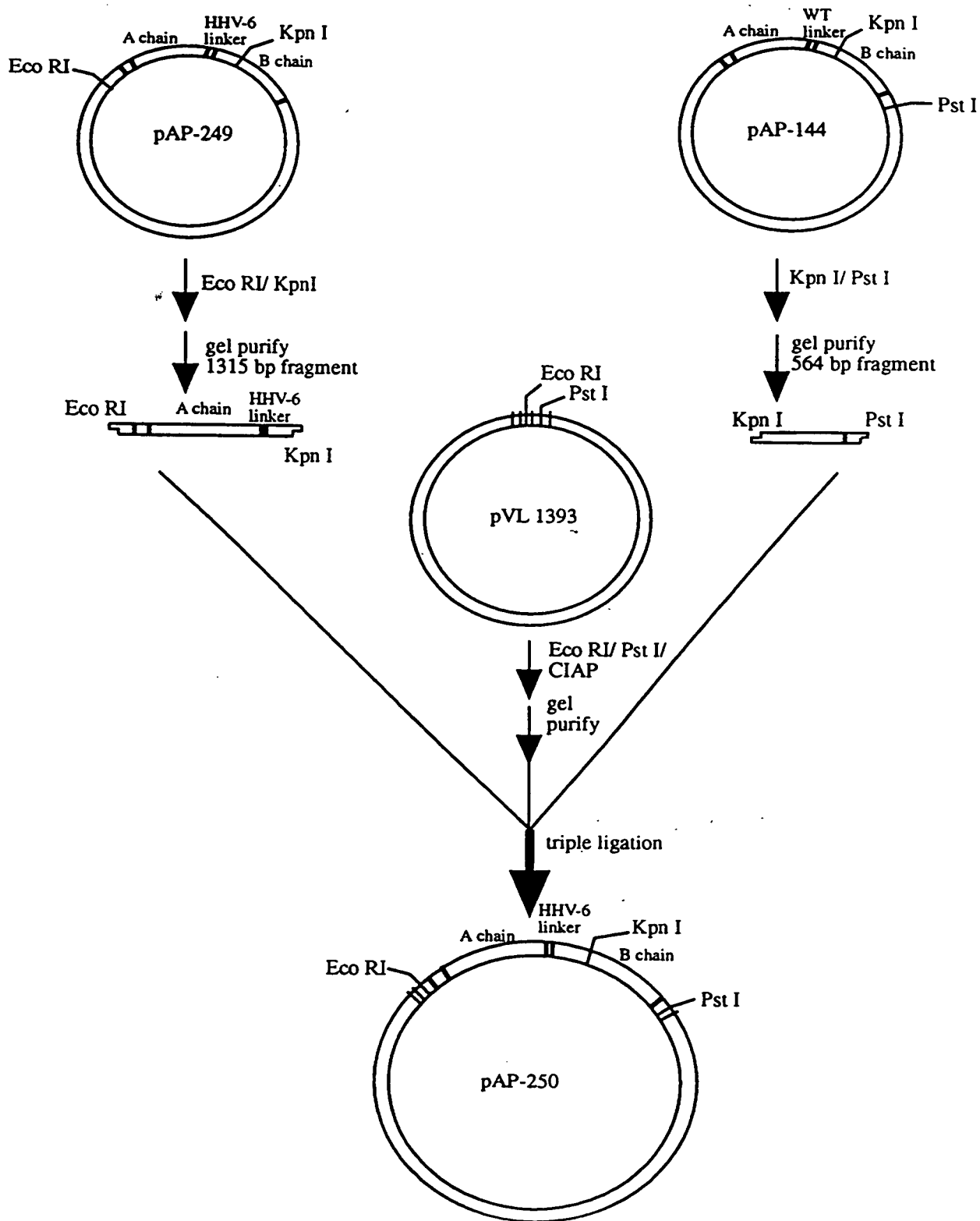
TC TTGCTTATAAGGCCAGTGGTGCCAAATTTAAT
AGAAACGAATATTCGGTCAACACGGTTTAAATTA
3' - ACCAGTGTCAAAAGAGCTAAATTTACGT -5'

primer HHV6-A2

PCR mutagenesis
ligate with pBluescript SK

pAP 249 linker
(HHV-6 variant)

TC TCGATTTTAAATGCATCGGTGCCAAATTTAAT
AGAGCTAAATTTACGTAGCCACGGTTTAAATTA



100/254

FIGURE 20D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTGCGCAA
AGCAAATGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTTCATCCTGACA
ACACCAGCCGATGGCAGCACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTAGTGAGTAGAAAAGTGACTACAAGTTTTA

451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTGTAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTCTCAGATTGGTTTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTTCGATTTTAAATGCATCGGTGCCAAATTTTAAATGC
AGCAGTGTCAAAGAAGCTAAAATTTACGTAGCCACGGTTTAAAATTACG

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101/254

FIGURE 20D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

0954151-041400

102/254

FIGURE 21

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-213/pAP-214 linker (Cathepsin B):

A chain- S L L K S R M V P N F N -B chain

pAP-215/pAP-216 linker (MMP-3):

A chain- R P K P Q Q F F G L M N -B chain

pAP-217/pAP-218 linker (MMP-7):

A chain- S L R P L A L W R S F N -B chain

pAP-219/pAP-220 linker (MMP-9):

A chain- S P Q G I A G Q R N F N -B chain

pAP-221/pAP-222 linker (THERMOLYSIN-LIKE MMP):

A chain- D V D E R D V R G F A S F L -B chain

pAP-241/pAP-242 linker (EBV-A):

A chain- S K L V Q A S A S G V N -B chain

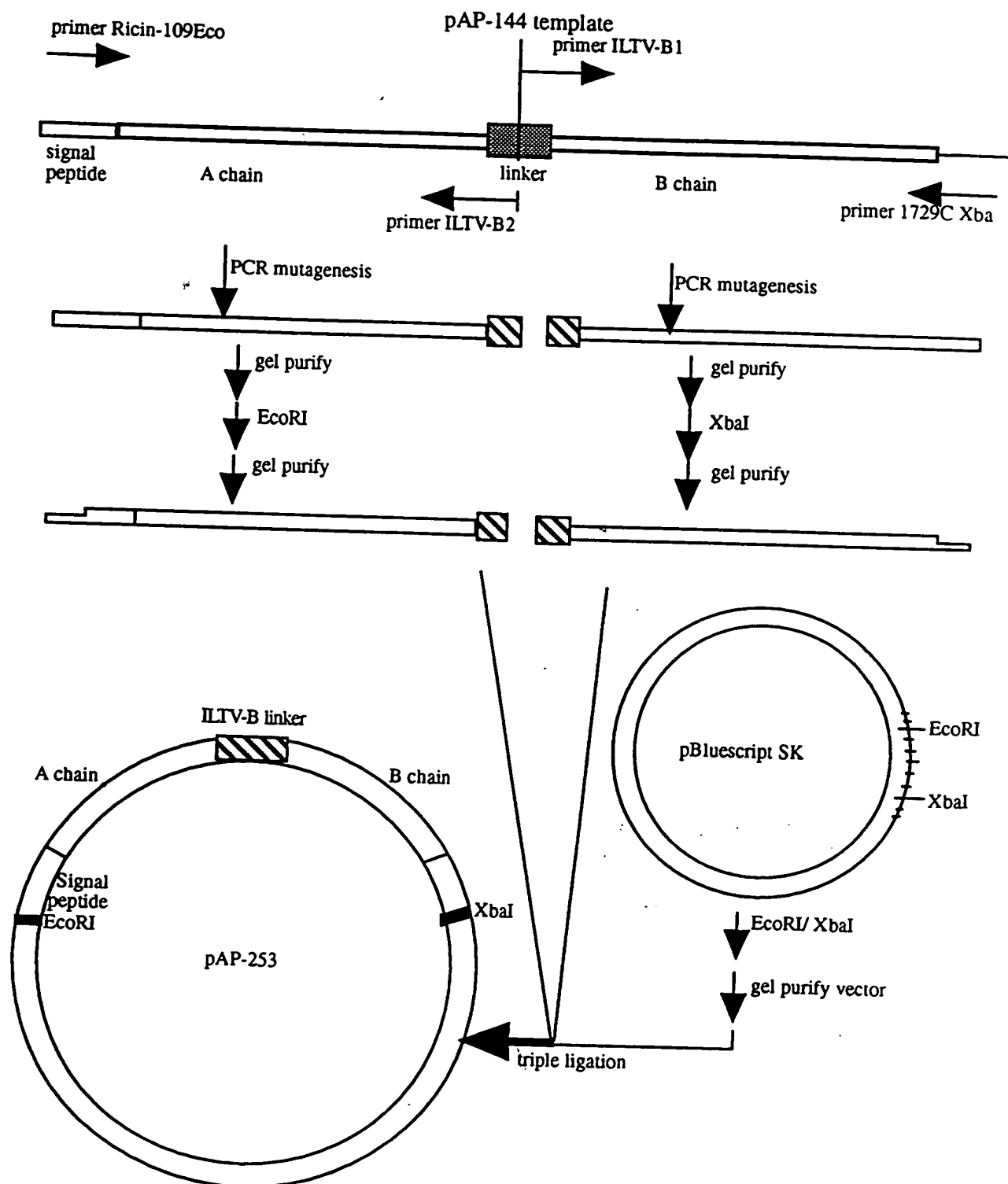
pAP-243/pAP-244 linker (EBV-B):

A chain- S S Y L K A S D A P D N -B chain

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103/254

FIGURE 22A



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FIGURE 22B

WT preprorin linker

primer ILTV-B1

5' - AATGAGGTAATTAATAATGCTGATGTTGT -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTAAT
 AGAAACGAAATATTCGGTCACCCACGGTTAAATTA

3' - AGCAGTGTCAAAAGATTCATAGATGTCCGT-5'

primer ILTV-B2

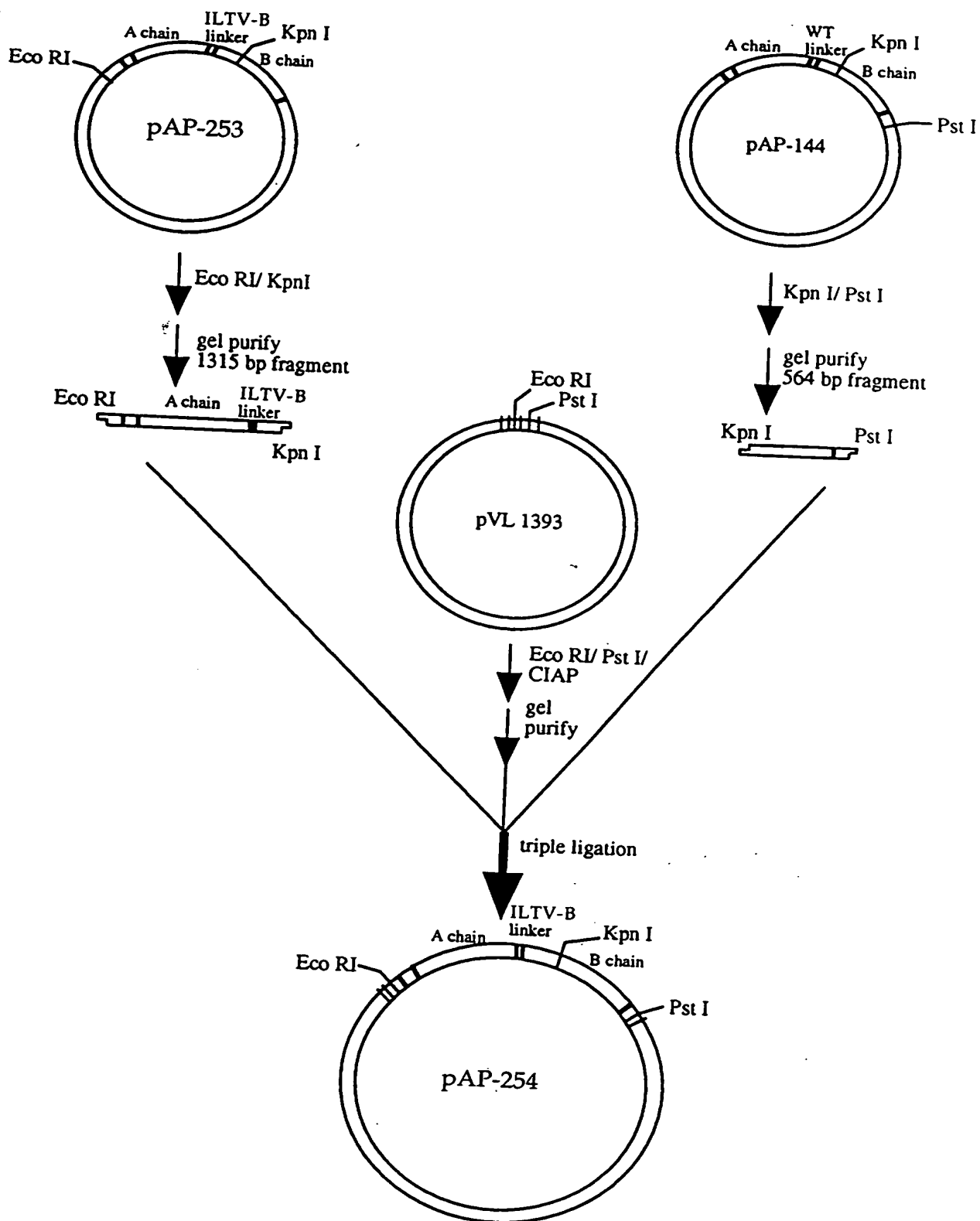
↓ PCR mutagenesis
 ↓ ligate with pBluescript SK

pAP 253 linker
 (ILTV-B variant)

TCTAAGTATCTACAGGCAATGAGGTAATTAATAAT
 AGATTCATAGATGTCCGTTTACTCCATTAAATGATTA

105/254

FIGURE 22C



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106/254

FIGURE 22D

10 20 30 40 50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTGCGAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCCTGTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTAA
451 CGATATACATTTCGCCCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG
GACCGAGCAAGGAAATATTAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCCTCGGAAACGATCAGGTTA
801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
901 TCGTCACAGTTTCTAAGTATCTACAGGCAAATGAGGTAATTACTAATGC
AGCAGTGTCAAAAGATTCATAGATGTCCGTTTACTCCATTAATGATTACG

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107/254

FIGURE 22D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGC GTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTT CAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTT CACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGT CAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTT CATACTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTC TTG CAGTGTGTGTCTG C CATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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108/254

FIGURE 23A

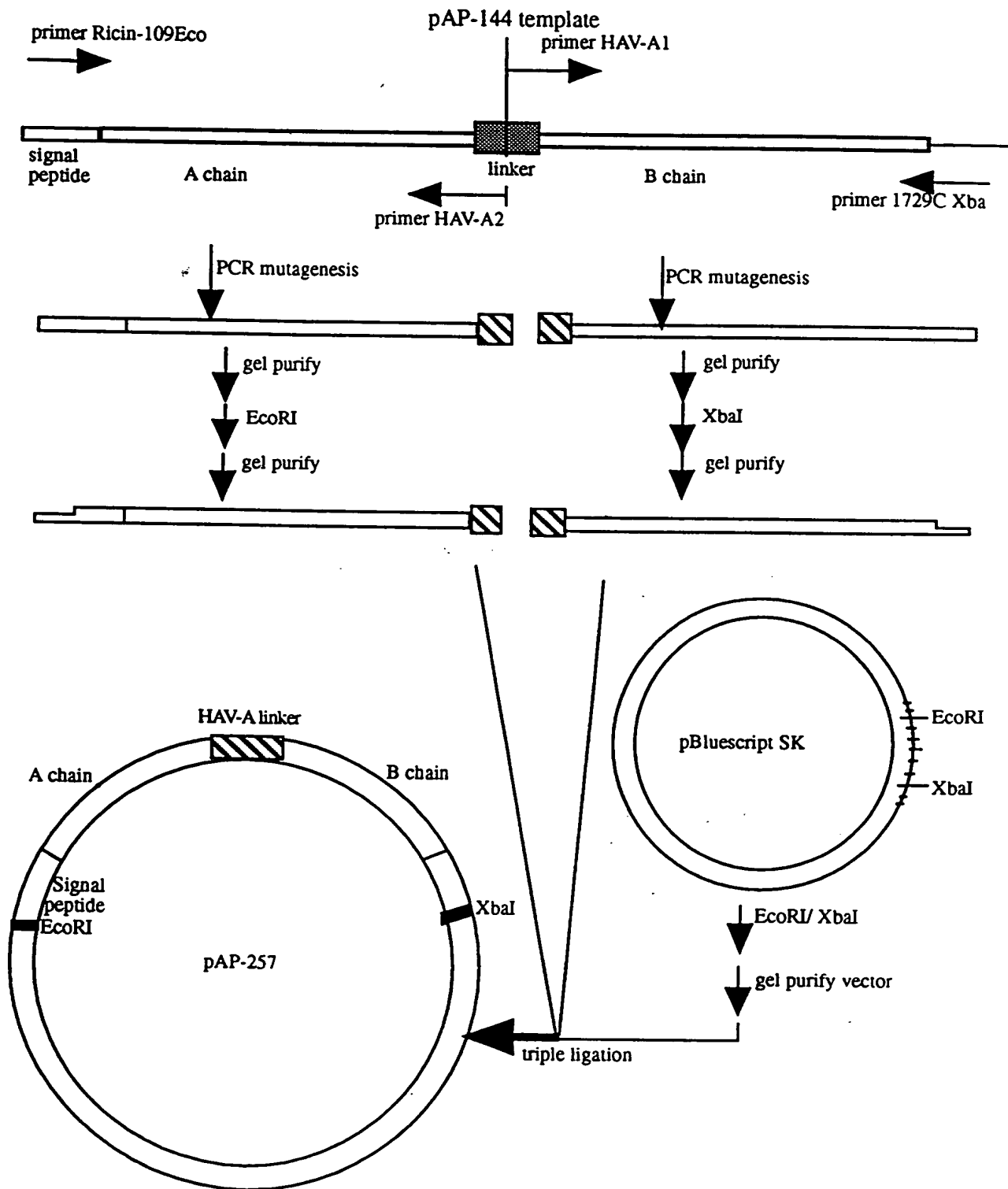


FIGURE 23B

WT preprorin linker

primer HAV-A1

5' - TCGTCTCTCAAATGGAATGCTGATGTTGT -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTAAT
AGAAACGAATATTCGGTACCCACGGTTAAATTA

3' - AGCAGTGTCAAAAGACTCGAATCTTGCGTT-5'

primer HAV-A2

PCR mutagenesis

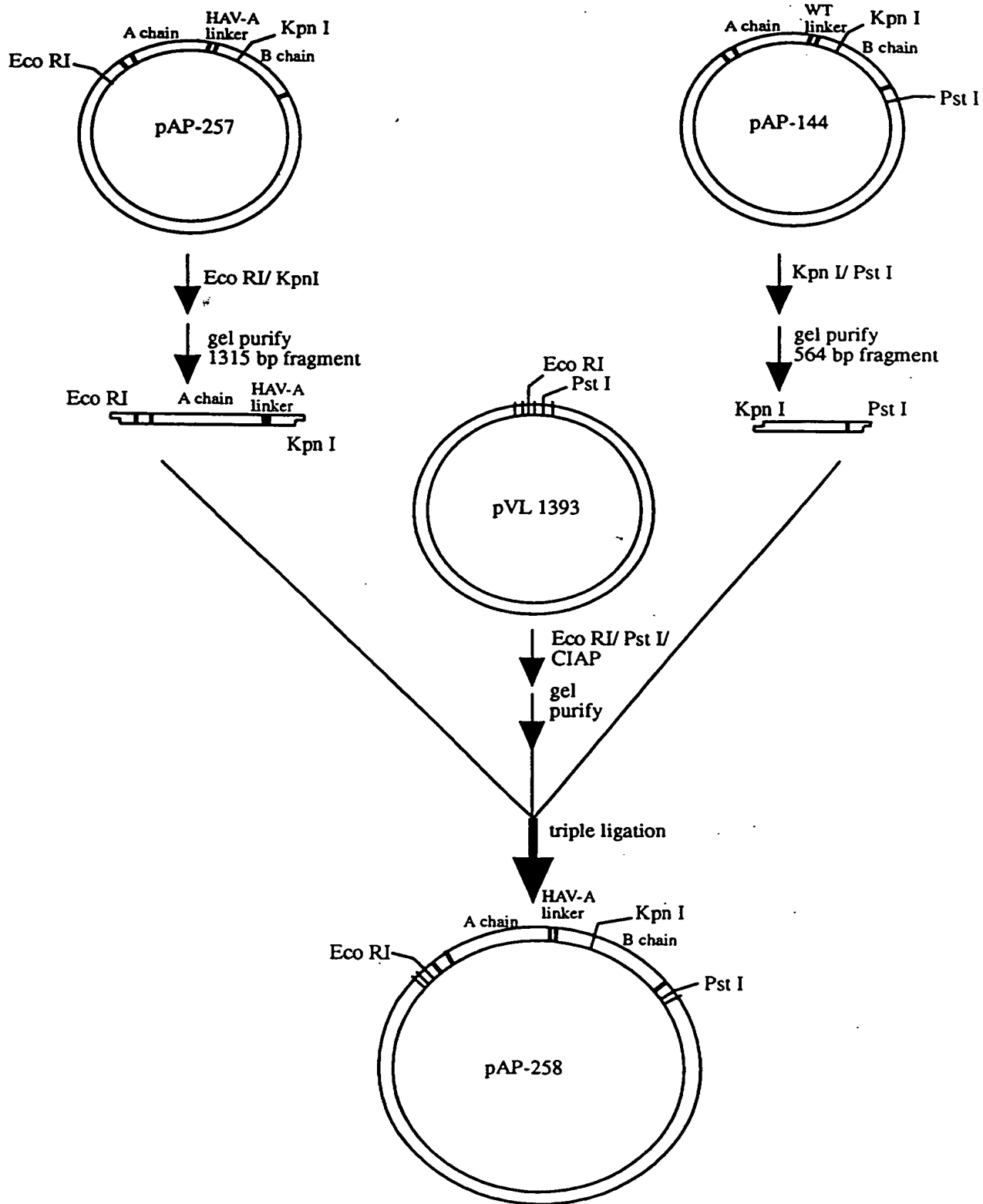
ligate with pBluescript SK

pAP 257 linker
(HAV-A variant)

TCTGAGCTTAGAACGCAATCGTCTCTCAAATGGAAT
AGACTCGAATCTTGCGTTAGCAAGAGTTTAAACCTTA

110/254

FIGURE 23C



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111/254

FIGURE 23D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA

451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTGTAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTTGGT

901 TCGTCACAGTTTTCTGAGCTTAGAACGCAATCGTTCTCAAATTGGAATGC
AGCAGTGTCAAAAGACTCGAATCTTGCGTTAGCAAGAGTTTAACCTTACG

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112/254

FIGURE 23D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGC GTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTT CAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGT TTAATACTTACG
CTTTCTCTGTTATGATAAGCTAGATTACCTTT CACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTG GCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGT TTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGT CACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTG GTTGTAATAACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGT TACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTT CATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTG CACCCGAGAAATACGTCACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTCTCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAC TGAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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113/254

FIGURE 24A

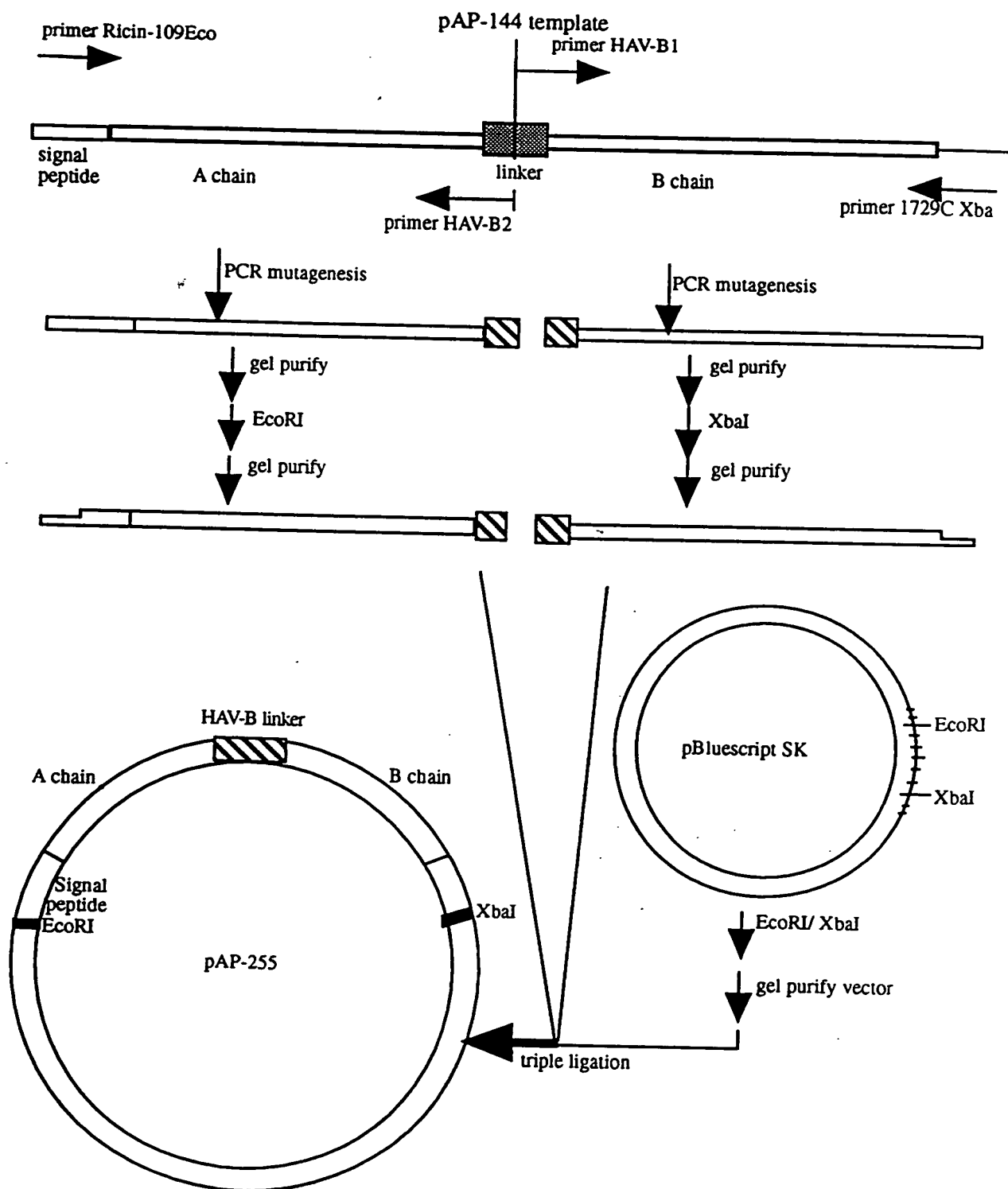


FIGURE 24B

WT preproricin linker

primer HAV-B1

5' - GGGATCGATGATGATAATGCTGATGTTGT -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCGGTCACCCACGGTTTAAATTA

3' - AGCAGTGTCAAAGACTCGAAACCCAGCGTT-5'

primer HAV-B2

PCR mutagenesis

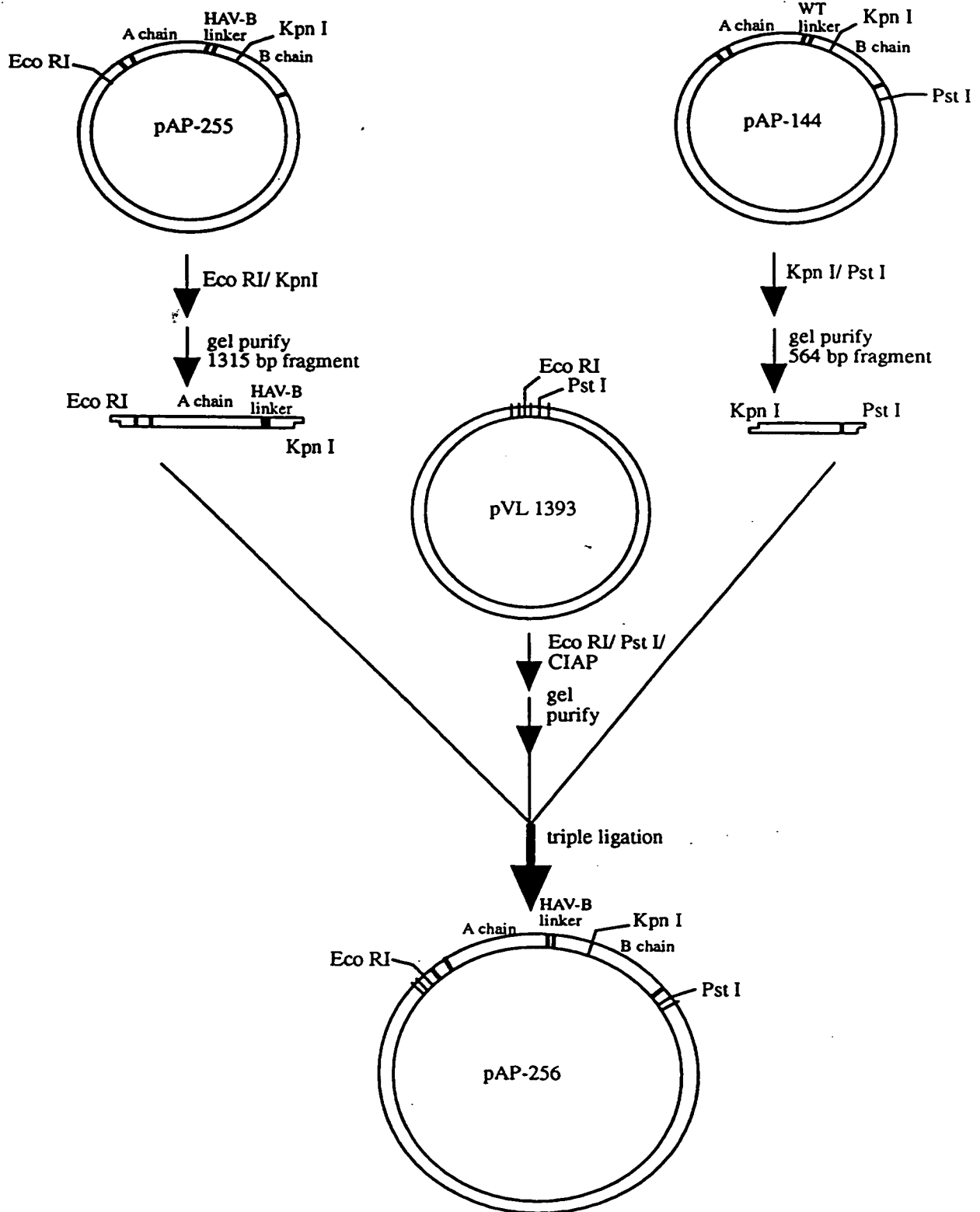
ligate with pBluescript SK

pAP 255 linker
(HAV-B variant)

TCTGAGCTTTGGTCGCAAGGATCGATGATAAT
AGACTCGAAACCCAGCGTTCCCTAGCTACTACTATA

115/254

FIGURE 24C



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116/254

FIGURE 24D

10 20 30 40 50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTTGTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC

201 TCGTTTAAACAACTGGAGCTGATGTGAGACATGATATACCACTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTGTTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTAA

451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACCTTGC
GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTGTAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC

551 CTATCTCAGCGCTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTTCCTTTATAATTTGCATCCAAATGATTTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTCTCAGATTGGTTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTCTGAGCTTTGGTCGCAAGGGATCGATGATGATAATGC
AGCAGTGTCAAAAGACTCGAAACCAGCGTTCCCTAGCTACTACTATTACG

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FIGURE 24D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCCTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCCTTGGCCTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTGTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTCTGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

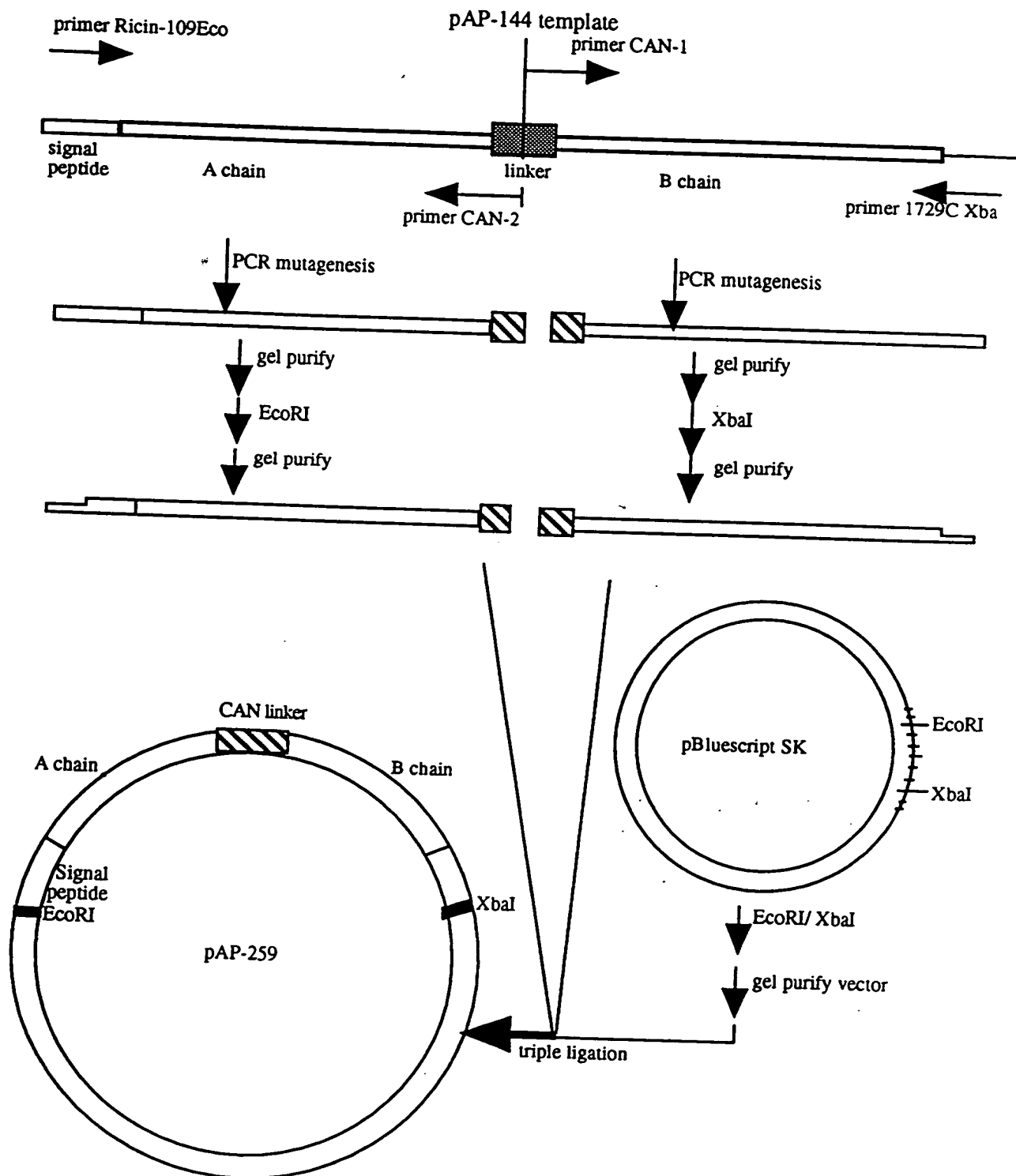
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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118/254

FIGURE 25A



0955151-04400

FIGURE 25B

WT preprorin linker

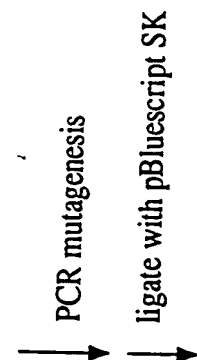
primer CAN-1

5' - TTCAGGCTAAATTTTAATGCTGAT -3'

TCTTTGCTTATAAGGCCAGTGCGTGCCAAATTTTAAT
AGAAACGAATATTCCGGTCACCCACGGTTTAAATTA

3' - AGCAGTGTCAAAAAGATTTCGGACGTTTCAAG-5'

primer CAN-2

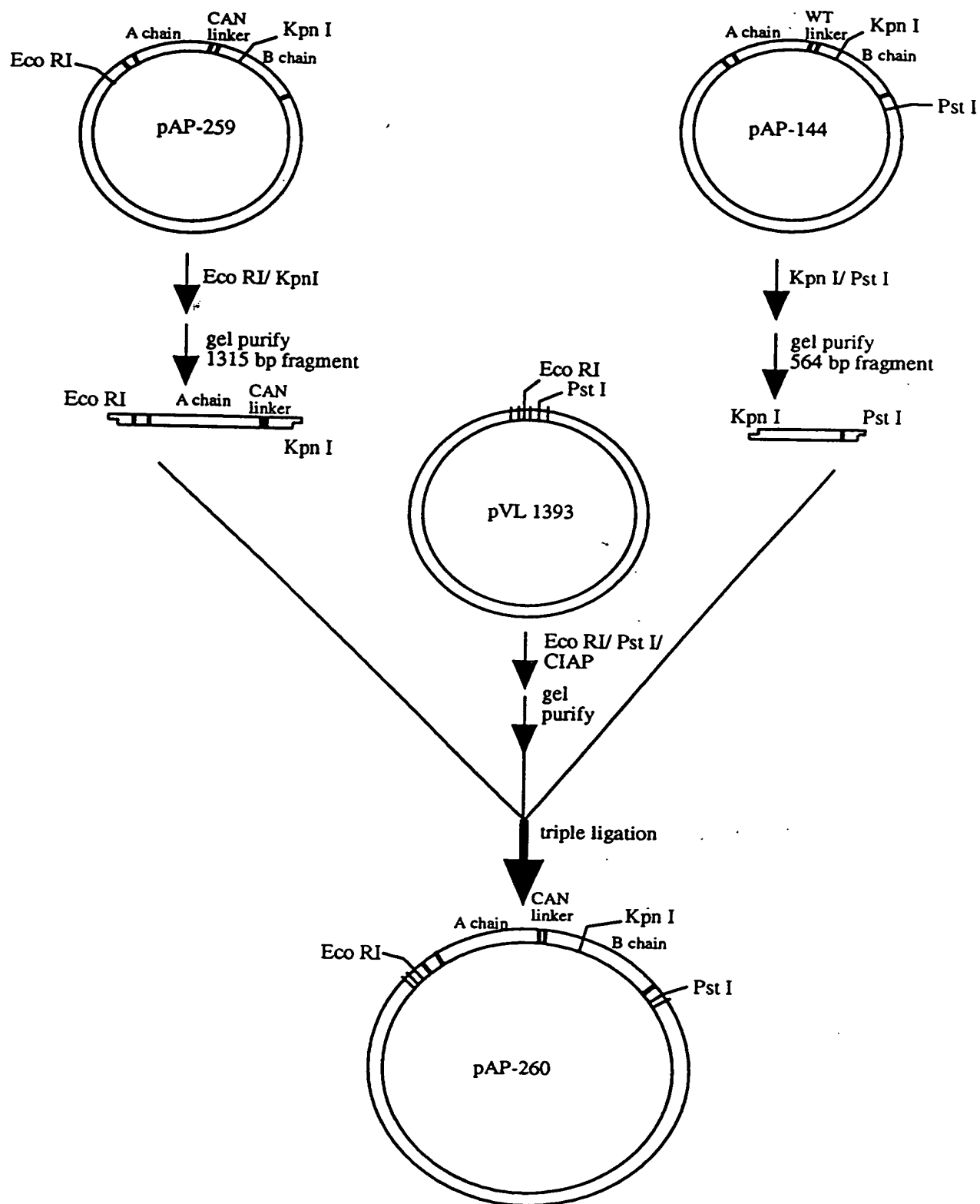


pAP 259 linker
(CAN variant)

TCTAAGCCTGCAAAAGTTCTTCAGGCTAAATTTTAAT
AGATTCGGACGTTTCAAGAAAGTCCGATTAAATTA

120/254

FIGURE 25C



00440-151550

121/254

FIGURE 25D

	10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA				
51	GGCAACATGGCTTTGTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG				
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC				
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
	TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT				
151	GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG				
	CGCCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC				
201	TCGTTTAAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTCGCAA				
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251	ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA				
	TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT				
301	AATCATGCAGAGCTTTCGTGTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351	TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA				
	ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT				
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT				
	TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTT				
451	CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC				
	GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTGTAACG				
501	TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC				
551	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601	CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG				
	GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT				
651	ATTCCAATATATTGAGGGAGAAAATGCGCACGAGAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT				
701	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA				
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTTTATCAACCCCTCT				
751	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT				
	GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCCTCGGAAACGATCAGGTTA				
801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA				
	AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTTACACTCAT				
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGAGGTTGGT				
901	TCGTCACAGTTTTCTAAGCCTGCAAAGTTCTTCAGGCTAAATTTTAATGC				
	AGCAGTGTCAAAGATTTCGGACGTTTCAAGAAGTCCGATTTAAAATTACG				

0955151.04.400

122/254

FIGURE 25D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCCTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAAGTTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTGTACAACCATGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAAACAATGTTGGTAACAACECGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCCTCAG
TCCGACTTGTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCCTCTCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGACAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTGCTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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123/254

FIGURE 26

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-223/224 linker (MAL-A):

A chain- Q V V Q L Q N Y D E E D -B chain

pAP-225/226 linker (MAL-B):

A chain- L P I F G E S E D N D E -B chain

pAP-227/228 linker (MAL-C):

A chain- Q V V T G E A I S V T M -B chain

pAP-229/230 linker (MAL-D):

A chain- A L E R T F L S F P T N -B chain

pAP-231/pAP-232 linker (MAL-E):

A chain- K F Q D M L N I S Q H Q -B chain

00551.04.400

124/254

FIGURE 27

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-245/246 linker (CMV-A):

A chain- S G V V N A S C R L A N -B chain

pAP-247/248 linker (CMV-B):

A chain- S S Y V K A S V S P E N -B chain

pAP-233/234 linker (HERPES SIMPLEX-1 A):

A chain- S A L V N A S S A H V N -B chain

pAP-235/236 linker (HERPES SIMPLEX-1 B):

A chain- S T Y L Q-A S E K F K N -B chain

pAP-249/250 linker (HUMAN HERPES VIRUS-6):

A chain- S S I L N A S V P N F N -B chain

pAP-237/pAP-238 linker (VZV-A):

A chain- S Q D V N A V E A S S N -B chain

pAP-239/pAP-240 linker (VZV-B):

A chain- S V Y L Q A S T G Y G N -B chain

pAP-253/pAP-254 linker (ILV):

A chain- S K Y L Q A N E V I T N -B chain

pAP-255/pAP-256 linker (HAV-A):

A chain- S E L R T Q S F S N W N -B chain

pAP-257/pAP-258 linker (HAV-B):

A chain- S E L W S Q G I D D D N -B chain

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125/254

FIGURE 28

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-259/260 linker (CAP-A):

A chain- S K P A K F F R L N F N -B chain

pAP-261/262 linker (CAP-B):

A chain- S K P I E F F R L N F N -B chain

pAP-263/264 linker (CAP-C):

A chain- S K P A E F F A L N F N -B chain

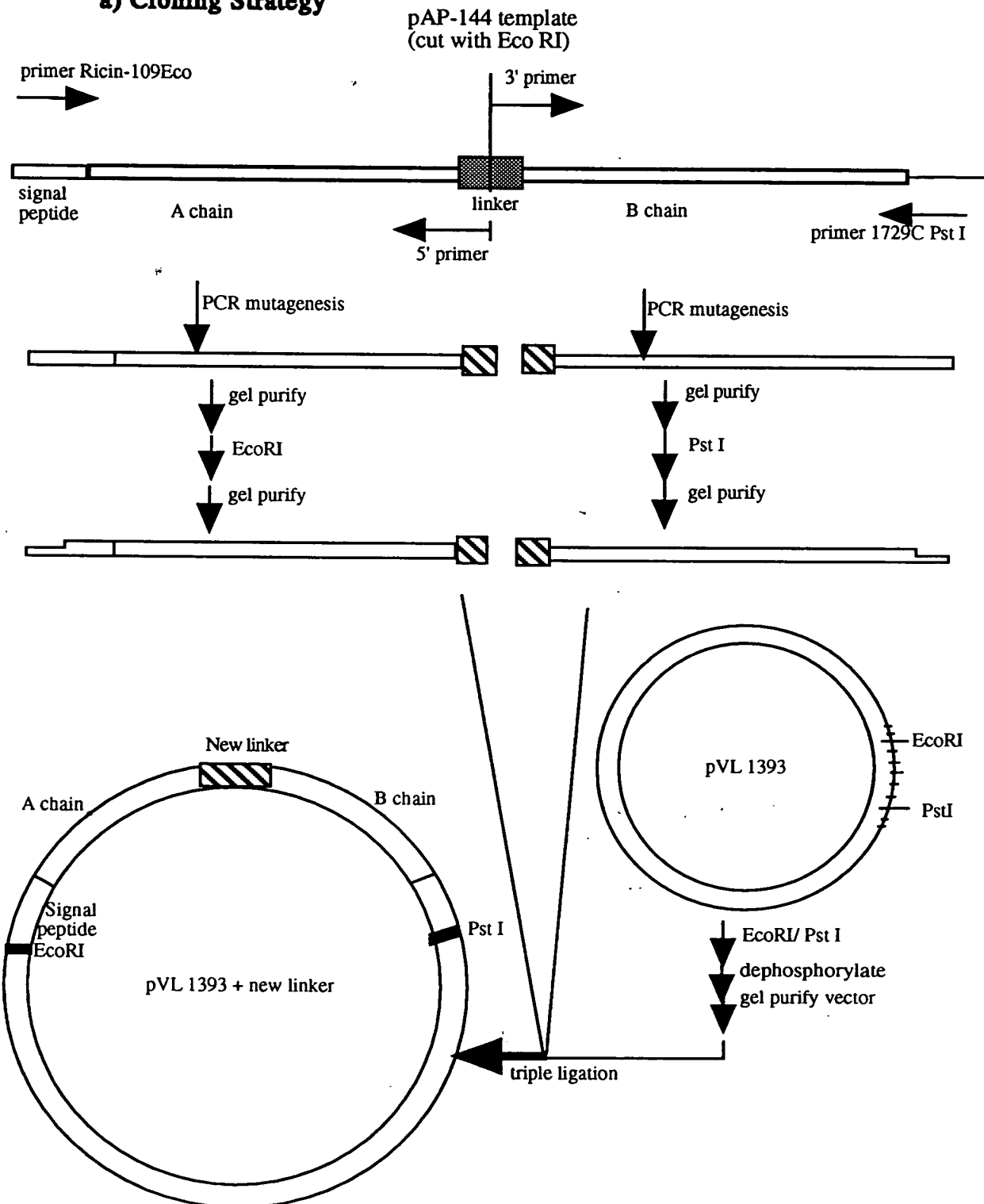
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126/254

FIGURE 29

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy

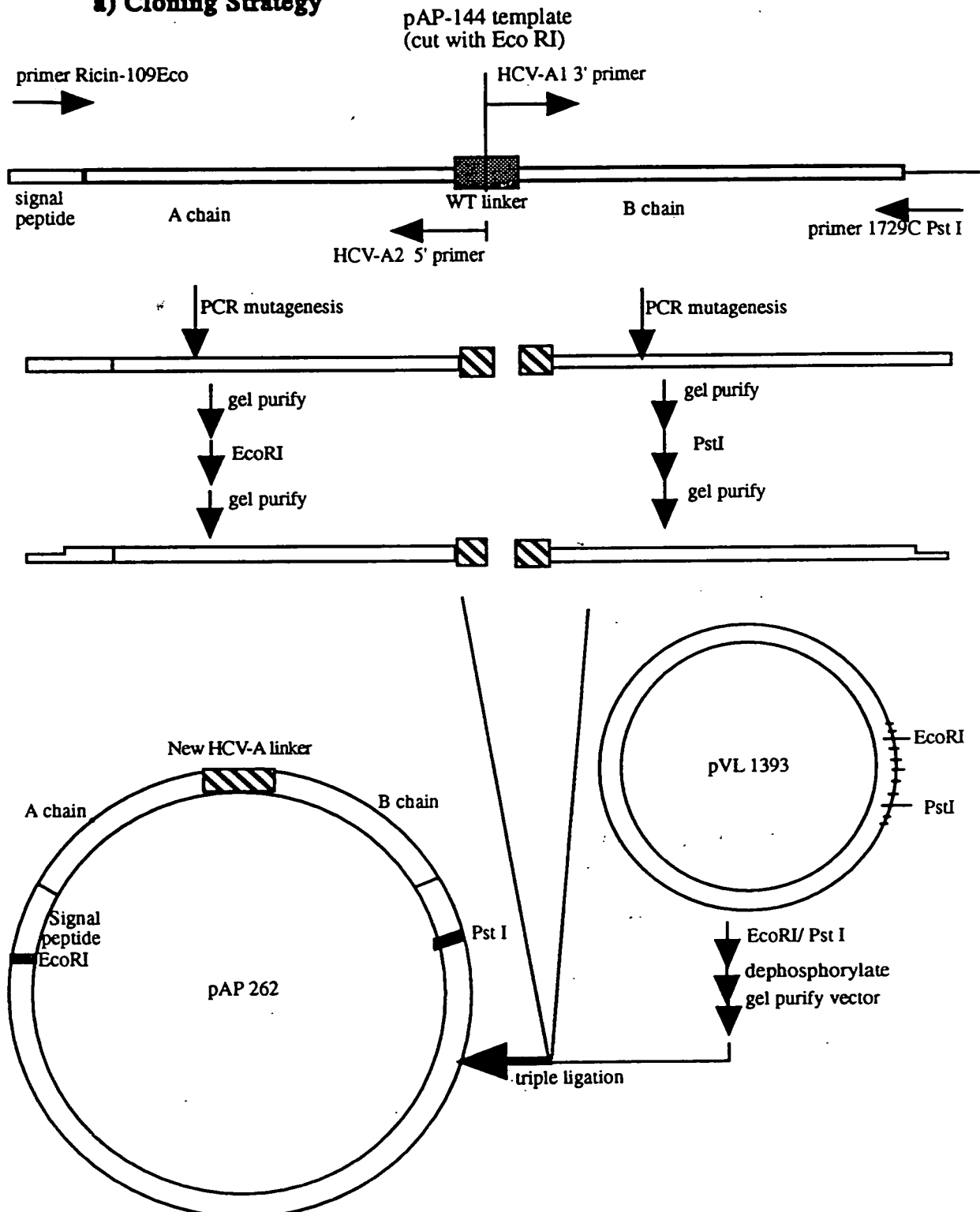


127/254

FIGURE 30A

PCR Mutagenesis of Preproricon Gene to Create An HCV-A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



00440"TSF560

FIGURE 30B

Sequence of HCV-A Linker Region

WT preprorin linker

primer HCV-A1

5' - TCGACATGGGTTTAAATGCTGATGTT -3'

TCCTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCGGTCAACCACGGTTTAAATTA

3' - GGTAGCAGTGTCAAACTAACCTCCATCACTGT-5'

5' primer HCV-A2

PCR mutagenesis

ligate with pVL1393

pAP 262 linker
(HCV-A variant)

GATTTGGAGGTAGTGACATCGACATGGGTTTAAAT
CTAAACCTCCATCACTGCTAGCTGTACCCAAAAATTA

129/254

FIGURE 30C (P1)

Sequence of pAP262 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATAACAGTGTGCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTGTTGCGCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

0951151-041400

130/254

FIGURE 30C (P2)

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTGATTTGGAGGTAGTGACATCGACATGGGTTTTTAATGC
AGCAGTGTCAAACATAAECTCCATCACTGTAGCTGTACCCAAAAATTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACATAAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

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131/254

FIGURE 30C (P3)

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA
1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP262

09551151-04400

132/254

FIGURE 30D

**-Amino Acid Sequence Comparison of Mutant
Preproricin Linker Region of HCV-A to Wild Type**

Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-262 linker: A chain- D L E V V T S T W V F N -B chain
(HCV-A linker)

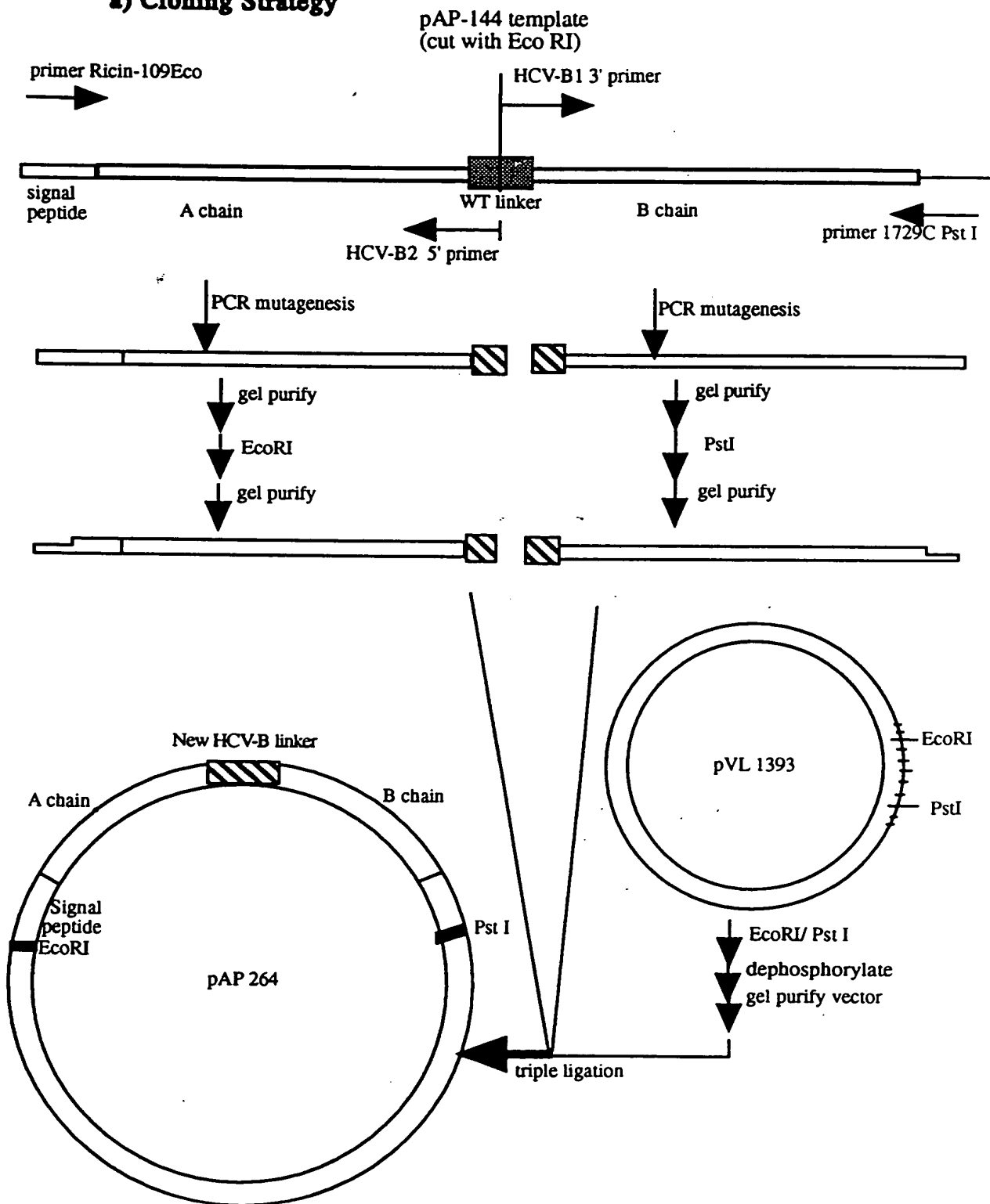
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004740" TESTS60

133/254

FIGURE 31A

PCR Mutagenesis of Preprorincin Gene to Create An HCV-B Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



004740" T5T5560

FIGURE 31B

Sequence of HCV-B Linker Region

WT preprorin linker

primer HCV-B1

5' - GCGTCACACCTTTTAAATGCTGATGTT -3'

TCCTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATCCGGTCACCCACGGTTTAAATTA

3' - GGTAGCAGTGTCAAACACTCTACCTTCTCACA-5'

5' primer HCV-B2

PCR mutagenesis

ligate with pVL1393

pAP 264 linker
(HCV-B variant)

GATGAGATGGAAGAGTGTGCGTCACACCTTTTAAAT
CTACTCTACCTTCTCACACCGCAGTGTGGAAATTA

135/254

FIGURE 31C (P1)

Sequence of pAP264 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

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136/254

FIGURE 31C (P2)

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTGTATGAGATGGAAGAGTGTGCGTCACACCTTTTAAATGC
AGCAGTGTCAAACACTCTACCTTCTCACACGCAGTGTGGAAAAATTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCATCGTAGGTGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACATAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTGTTTATCACCTGTTTATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

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137/254

FIGURE 31C (P3)

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP264

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138/254

FIGURE 31D

**-Amino Acid Sequence Comparison of Mutant
Preproricin Linker Region of HCV-B to Wild Type**

Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-264 linker: A chain- D E M E E C A S H L F N -B chain
(HCV-B linker)

004740 TSTTS60

FIGURE 32A

- PCR Mutagenesis of Preproricin Gene to Create An HCV-C Variant Gene in Baculovirus Transfer Vector, pVL 1393

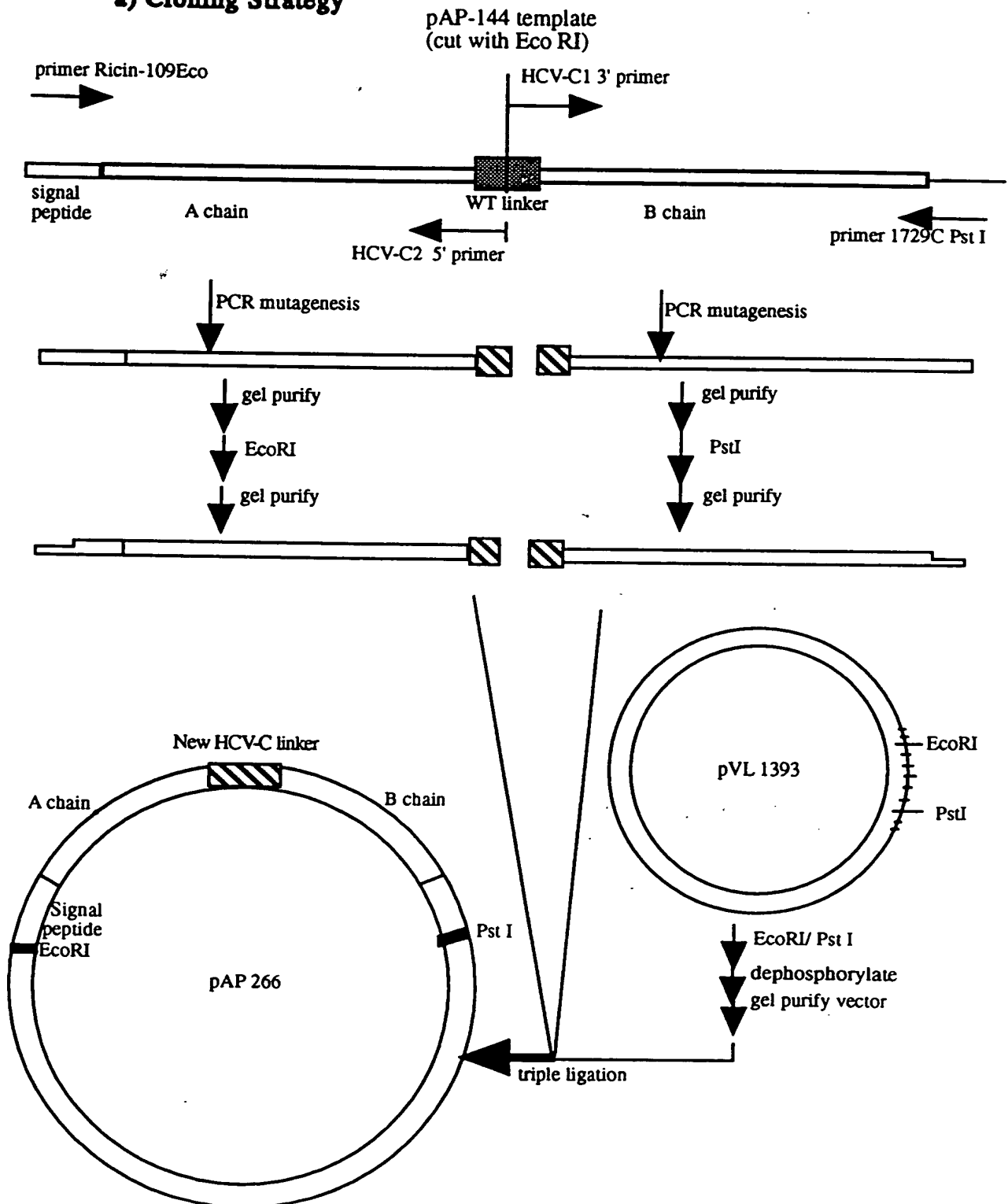
a) Cloning Strategy

FIGURE 32B

Sequence of HCV-C Linker Region

WT preprorin linker

primer HCV-C1

5' - TCGATGTCATATTTTAATGCTGATGTT - 3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAATATTCCTGGTCACCCACGGTTAAATTA

3' - GGTAGCAGTGTCAAACTCCTGCAACATACACA - 5'

5' primer HCV-C2

PCR mutagenesis

ligate with pVL1393

pAP 266 linker
(HCV-C variant)

GAGGACGTTGTATGTTGTCGATGTCATATTTTAAT
CTCCTGCAACATACACAAGCTACAGTATATAATTA

141/254

FIGURE 32C (P1)

Sequence of pAP266 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTTCATCCTGACA
ACACCAGCCGATGGCAGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
TATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAACGTAGGTTTACTAAAGTCTTCGTCGTTCT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

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142/254

FIGURE 32C (P2)

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTGAGGACGTTGTATGTTGTTTCGATGTCATATTTTAATGC
AGCAGTGTCAAACCTCCTGCAACATACAACAAGCTACAGTATAAAATTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTGCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTTACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACCAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

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143/254

FIGURE 32C (P3)

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCA GTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTGAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP266

0044075560

144/254

FIGURE 32D

**-Amino Acid Sequence Comparison of Mutant
Preproricin Linker Region of HCV-C to Wild Type**

Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-266 linker: A chain- E D V V C C S M S Y F N -B chain
(HCV-C linker)

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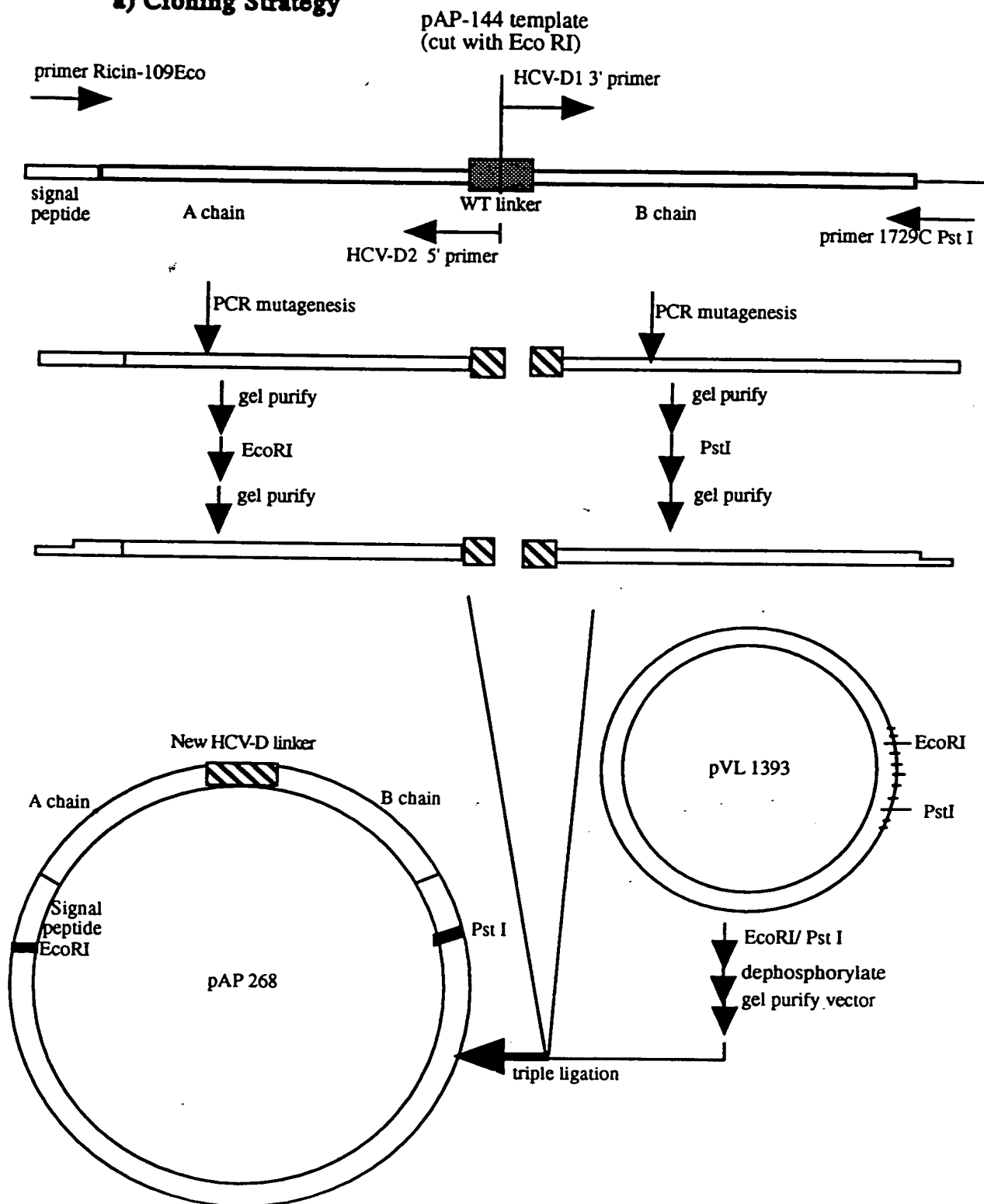
FIGURE 33A**PCR Mutagenesis of Preprorincin Gene to Create An HCV-D Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

FIGURE 33B

Sequence of HCV-D Linker Region

WT preprorcin linker

primer HCV-D1

5' - GCGCCAATAACTGCTTATGCTGATGTTGTATG -3'

TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT
AGAAACGAAATATTCGGTACCCACGGTTTAAATA

3' - GGTAGCAGTGTCAAATTCCCCACCTCTAACGAT-5'

5' primer HCV-D2

PCR mutagenesis

ligate with pVL1393

pAP 268 linker
(HCV-D variant)

AAGGGTGGAGATTGCTAGCGCCAATAACTGCTTAT
TTCCCCACCTCTAACGATCGGGTTATTGACGAATA

147/254

FIGURE 33C (P1)

Sequence of pAP268 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTCCGGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACTGAGCTGATGTGAGACATGATATAACAGTGTTCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451 CGATATACATTTCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

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148/254

FIGURE 33C (P2)

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTAAGGGGTGGAGATTGCTAGCGCCAATAACTGCTTATGC
AGCAGTGTCAAATTCGCCACCTCTAACGATCGCGGTTATTGACGAATACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACATAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAAACCATGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

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149/254

FIGURE 33C (P3)

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP268

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150/254

FIGURE 33D

**-Amino Acid Sequence Comparison of Mutant
Preproricin Linker Region of HCV-D to Wild Type**

Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-268 linker: A chain- K G W R L L A P I T A Y -B chain
(HCV-D linker)

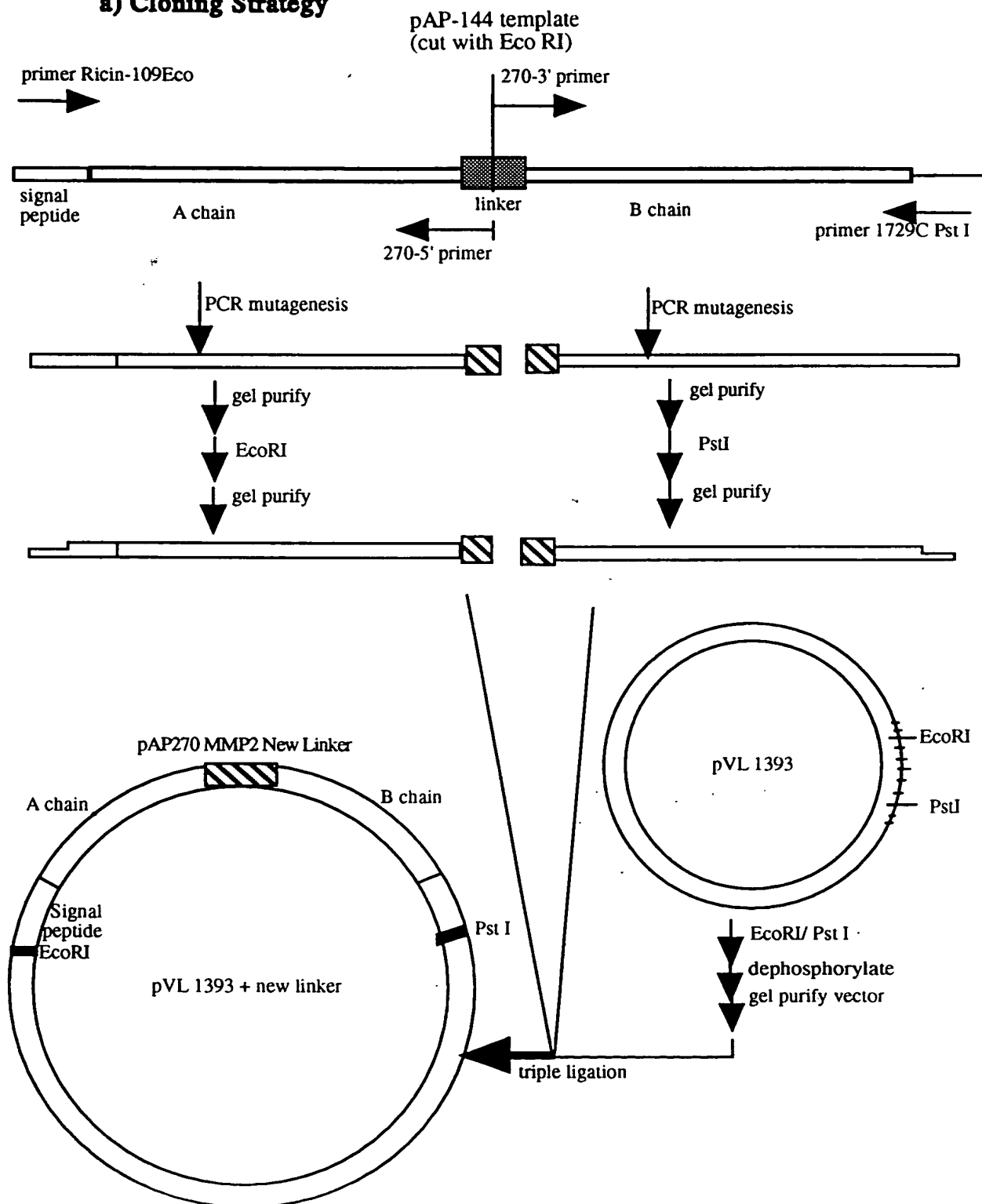
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151/254

FIGURE 34A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



152/254

FIGURE 34B

Sequence of MMP-2 Linker Region

WT preprocin linker

primer 270-3'

5' - TGGGCTCCTAATTTTAATGCTGATGTTTGT - 3'

| ** ** *

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAAATTA-----

*** ** *

3' - AGCAGTGTCAAAAGAAACGCGGACCCAAAT - 5'

primer 270-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 270 linker

(MMP-2 variant)

-----TCTTTGCCCCCTGGGTTTA|TGGGCTCCTAATTTTAAT-----
-----AGAAACGCGGACCCAAAT|ACCCGAGGATTAAAATTA-----

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153/254

FIGURE 34C (P1)

Sequence of pAP270 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATAACAGTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTAA
451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAACGTTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

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154/254

FIGURE 34C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTTTGCCCCTGGGTTTATGGGCTCCTAATTTTAATGC
AGCAGTGTCAAAGAAACGGGGACCCAAATACCCGAGGATTAAAATTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTGAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

0055451.044400

155/254

FIGURE 34C (P3)

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTGAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP270

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156/254

FIGURE 34D

**Amino acid sequence Comparison of Mutant Preproricin Linker
region of MMP-2 to Wild Type**

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-270 (MMP-2) linker: A chain- S L P L G L W A P N F N -B chain

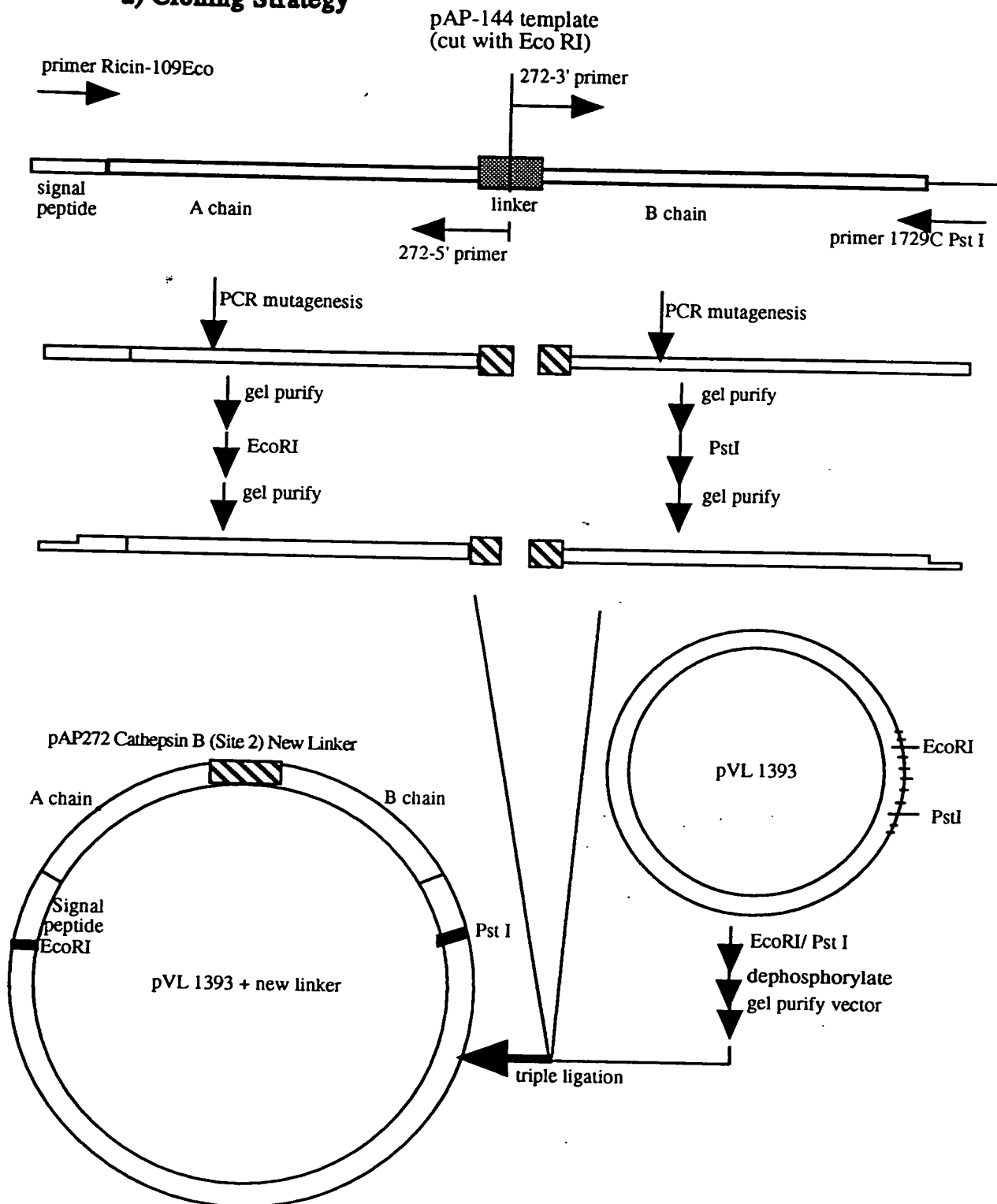
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157/254

FIGURE 35A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



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158/254

FIGURE 35B

Sequence of Cathepsin B (Site 2) Linker Region

WT preprocin linker

```

      primer 272-3'
      5' - AGGATGCCAAATTTTAATGCTGATGTTTGT -3'
              | * * *
-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCGGT|CACCATGGTTTAAAATTA-----
              *****
3' -AGCAGTGTCAAAGAAACGAATATCGATCT -5'
      primer 272-5'
  
```

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 272 linker
(Cathepsin B Site 2 variant)

```

-----TCTTTGCTTATAGCTAGA|AGGATGCCTAATTTTAAT-----
-----AGAAACGAATATCGATCT|TCCTACGGATTAAAATTA-----
  
```

159/254

FIGURE 35C (P1)

Sequence of pAP272 insert

	10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA				
51	GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG				
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC				
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
	TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT				
151	GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG				
	CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC				
201	TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATAACAGTGTTGCCAA				
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251	ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA				
	TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT				
301	AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351	TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA				
	ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT				
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT				
	TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA				
451	CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACCTTGC				
	GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG				
501	TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTCTTTTATAGCTCAACCCTTACCAGGTGATCTCCTCC				
551	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601	CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG				
	GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTC				
651	ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT				

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160/254

FIGURE 35C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTTTGCTTATAGCTAGAAGGATGCCTAATTTTAATGC
AGCAGTGTCAAAGAAAGGAATATCGATCTTCCTACGGATTAAAATTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTTACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCAACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

0044151.04400

161/254

FIGURE 35C (P3)

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP272

004440" TSTT560

162/254

FIGURE 35D

**Amino acid sequence Comparison of Mutant Preproricin Linker
region of Cathepsin B Site 2 to Wild Type**

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-272 (Cathepsin B 2) linker: A chain- S L L I A R R M P N F N -B chain

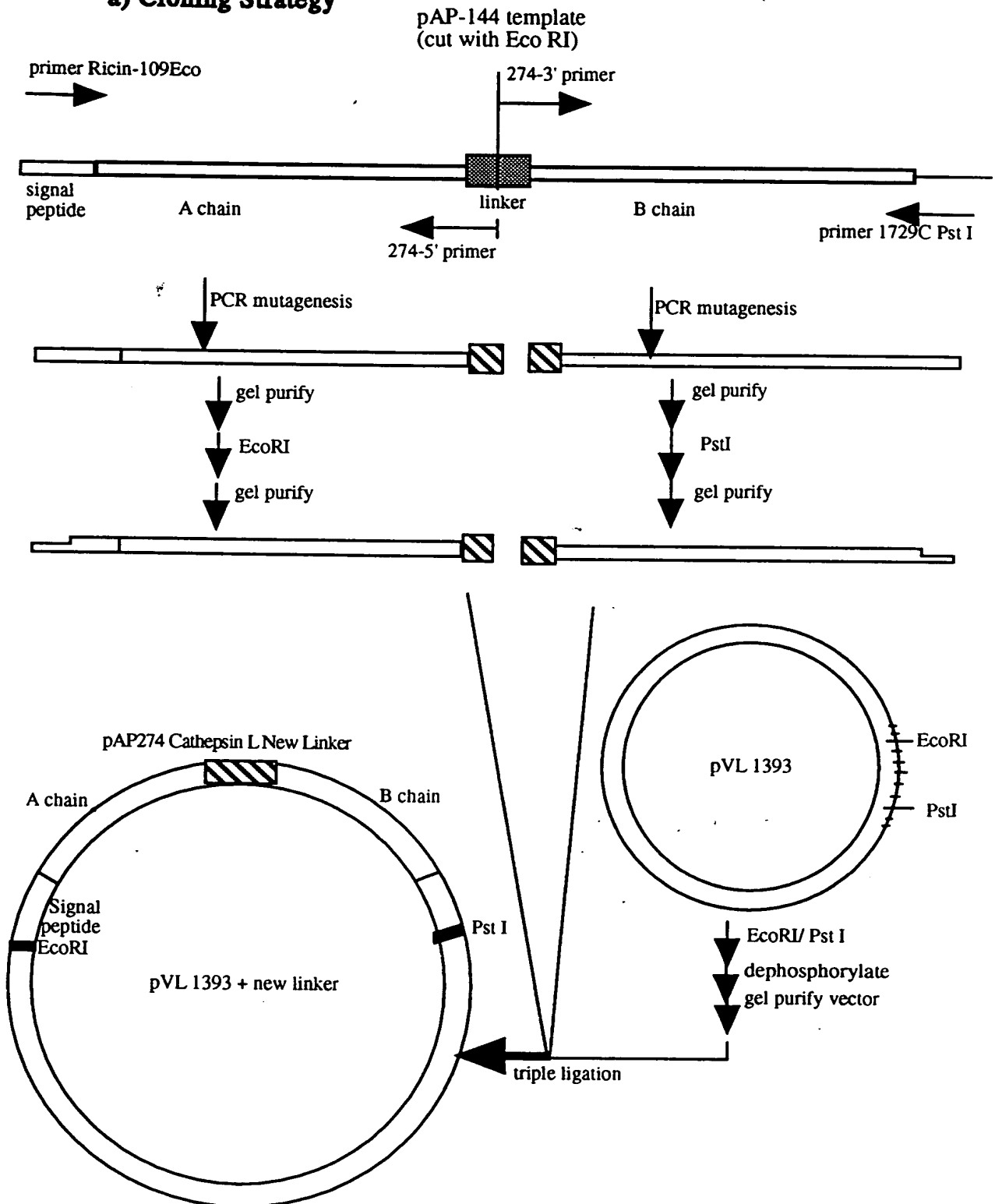
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163/254

FIGURE 36A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



164/254

FIGURE 36B

Sequence of Cathepsin L Linker Region

WT preprocin linker

primer 274-3'

5' - TCATGGGCTAATTTTAATGCTGATGTTTGT -3'

|***** *

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAAATTA-----

*** **

3' -AGCAGTGTCAAAAGAAACGAATATAAGGCC -5'

primer 274-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 274 linker

(Cathepsin L variant)

-----TCTTTGCTTATATTCCGG|TCATGGGCTAATTTTAAT-----
-----AGAAACGAATATAAGGCC|AGTACCCGATTAAAATTA-----

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165/254

FIGURE 36C (P1)

Sequence of pAP274 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC
101 AGGATAACAACATATTTCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTAA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTGTAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTC
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

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166/254

FIGURE 36C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTTTGCTTATATTCCGGTCATGGGCTAATTTTAATGC
AGCA^gTGTCAAAGAAAGGAATATAAGGCCAGTACCCGATTAAAATTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTGTTACAACCATGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

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167/254

FIGURE 36C (P3)

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCACTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP274

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168/254

FIGURE 36D

**Amino acid sequence Comparison of Mutant Preproricin Linker
region of Cathepsin L to Wild Type**

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-274 (Cathepsin L) linker: A chain- S L L I F R S W A N F N -B chain

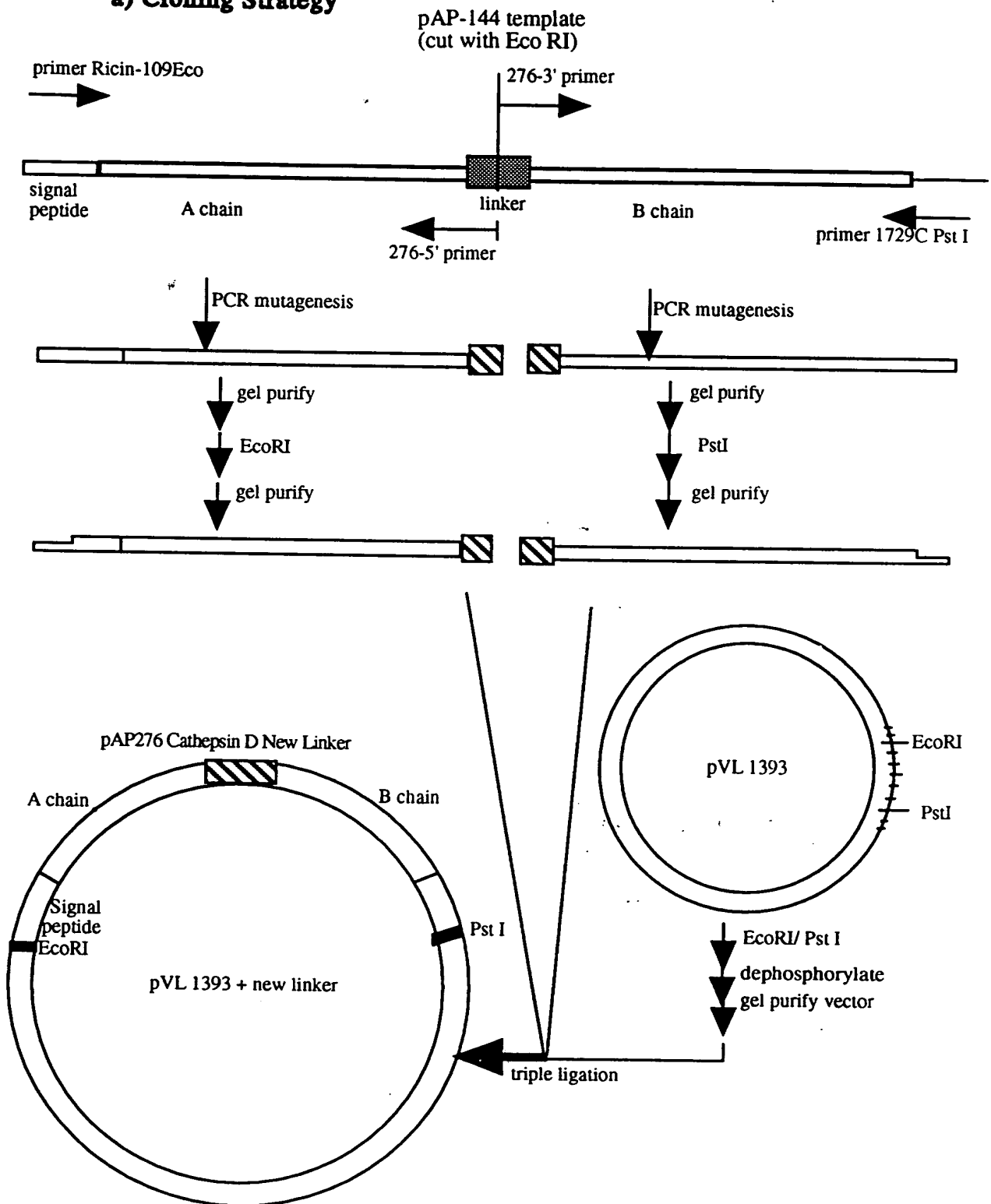
004400" TSTTSS60

169/254

FIGURE 37A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



170/254

FIGURE 37B

Sequence of Cathepsin D Linker Region

WT preprocin linker

primer 276-3'
5' - ACTGTTATTGTTATCACCGCTGATGTTTGT -3'
-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGATAACGAATATTCCGG|CACCATGGTTTAAAATTA-----
***** * * * *
3' -AGCAGTGTCAAAAGACCACAACAGTAGCGA -5'
primer 276-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 276 linker

(Cathepsin D variant)

-----TCTGGTGTGTCATCGCT|ACTGTTATTGTTATCACC-----
-----AGACCACAACAGTAGCGA|TGACAATAACAATAGTGG-----

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171/254

FIGURE 37C (P1)

Sequence of pAP276 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGCG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTTA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

004400" T5T560

172/254

FIGURE 37C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTGGTGTGTGCATCGCTACTGTTATTGTTATCACCGC
AGCA^gTGTCAAAGACCACAACAGTAGCGATGACAATAACAATAGTGCGC

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGA^tCTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

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173/254

FIGURE 37C (P3)

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP276

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174/254

FIGURE 37D

**Amino acid sequence Comparison of Mutant Preproricin Linker
region of Cathepsin D to Wild Type**

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain
pAP-276 (Cathepsin D) linker: A chain- S G V V I A T V I V I T -B chain

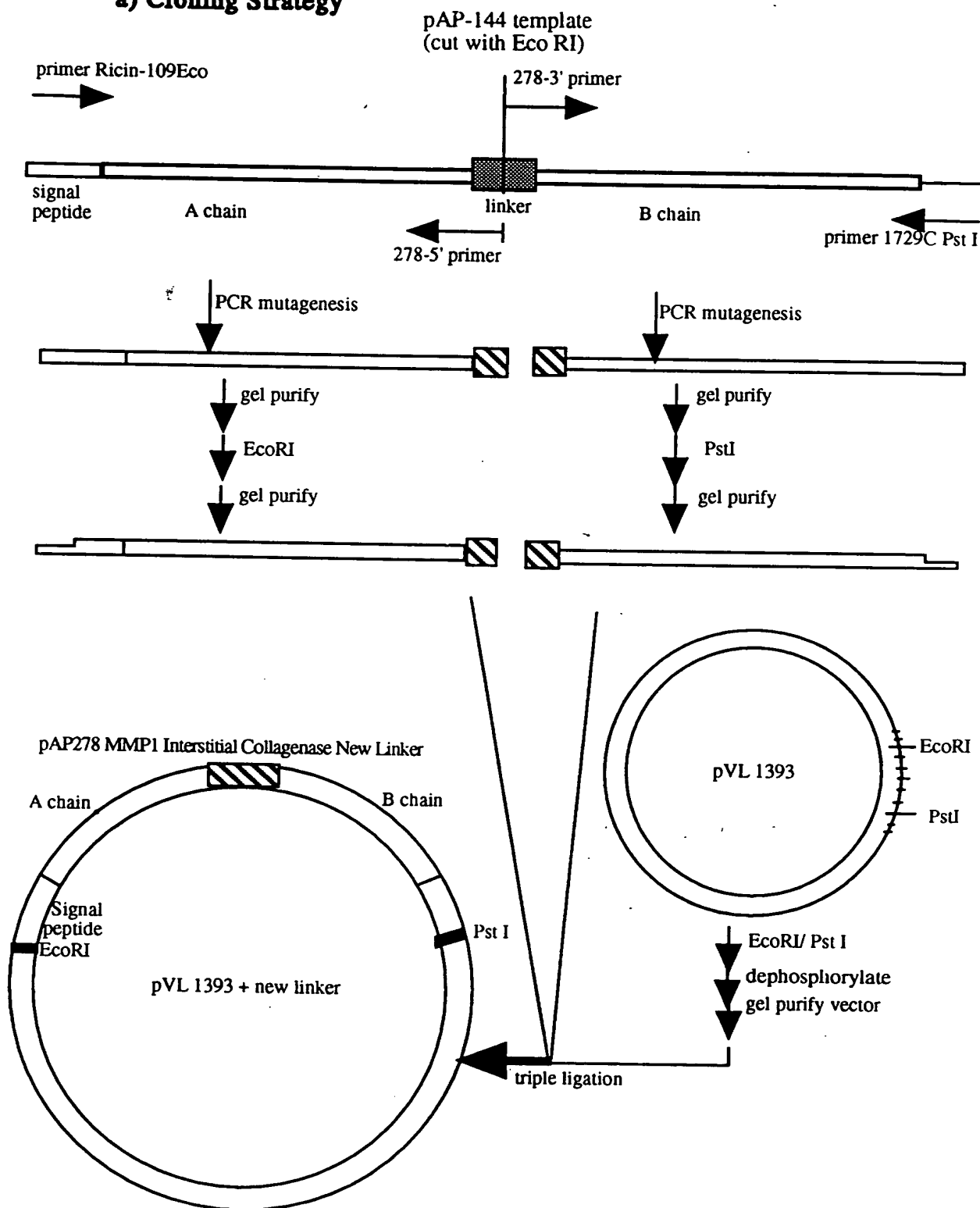
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175/254

FIGURE 38A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



176/254

FIGURE 38B

Sequence of MMP-1 (Interstitial collagenase) Linker Region

WT preprocin linker

primer 278-3'

5' - ATTTGGGGACAGTTTAATGCTGATGTTTGT - 3'

* * * * *

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAAATTA-----

** * * * * *

3' - AGCAGTGTCAAAAGAAACCCAGGAGTTCCG - 5'

primer 278-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 278 linker

(MMP-1 variant)

-----TCTTTGGGTCCTCAAGGC|ATTTGGGGACAGTTTAAT-----
-----AGAAACCCAGGAGTTCCG|TAAACCCCTGTCAAATTA-----

095151-044400

177/254

FIGURE 38C (P1)

Sequence of pAP278 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTGA
451 CGATATACATTGCGCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGAGCAGCAAG
GACCGAGCAAGGAAATATTAACGTTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

095151-041400

178/254

FIGURE 38C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTTTGGGTCTCAAGGCATTTGGGGACAGTTTAATGC
AGCAGTGTCAAAGAAACGCAGGAGTTCCGTAAACCCCTGTCAAATTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCATCGTAGGTGCAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGTTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTTACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

095151-041400

179/254

FIGURE 38C (P3)

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP278

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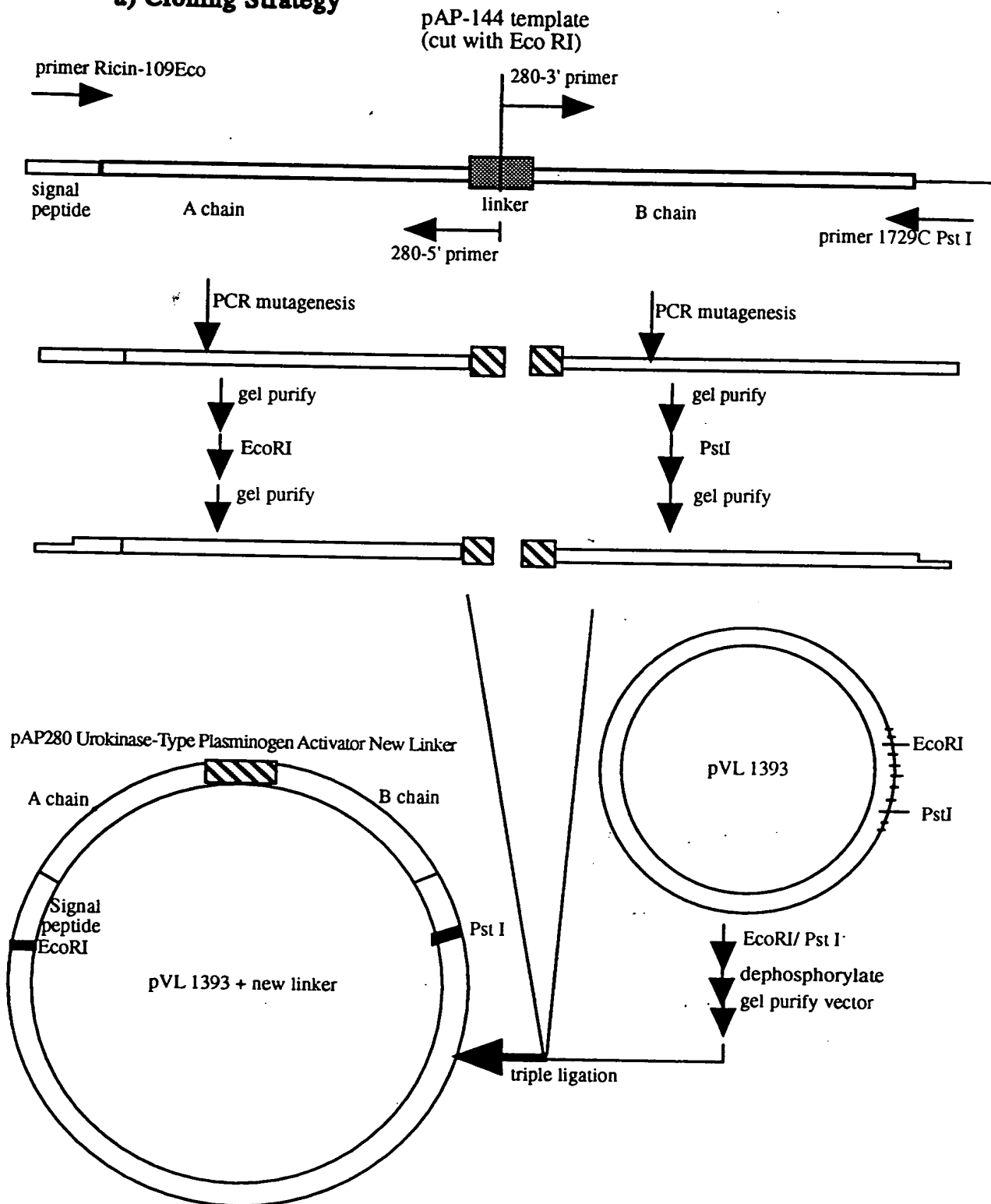
180/254

FIGURE 38D

Figure 38. d) Amino acid sequence Comparison of Mutant Preproricin Linker region of MMP-1 (Interstitial collagenase) to Wild Type

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain
pAP-278 (MMP-1) linker: A chain- S L G P Q G I W G Q F N -B chain

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FIGURE 39A**PCR Mutagenesis of Preprorincin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

182/254

FIGURE 39B

Sequence of Urokinase-Type Plasminogen Activator Linker Region

WT preprocin linker

primer 280-3'

5' - GTTGTCTGGTGGCTCTGTAGCTGATGTTTGT -3'

* * * * *

-----TCTTTGCTTATAAGGCCA | GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT | CACCATGGTTTAAAATTA-----
***** * * *

3' -AGCAGTGTCAAATTTTTTAGGGGACCTTCT -5'

primer 280-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 280 linker

(uPA variant)

-----AAAAAATCCCCTGGAAGA | GTTGTCTGGTGGCTCTGTA-----
-----TTTTTTAGGGGACCTTCT | CAACAGCCACCGAGACAT-----

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183/254

FIGURE 39C (P1)

Sequence of pAP280 insert

10 20 30 40 50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAAGTCAAGTTTGA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTGTAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCTGTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

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184/254

FIGURE 39C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTAAAAAATCCCCTGGAAGAGTTGTCGGTGGCTCTGTAGC
AGCAGTGTCAAATTTTTTAGGGGACCTTCTCAACAGCCACCGAGACATCG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTGTTTATCACCTGTTTATACCTATCTCCTGACATCGTCACTTT

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185/254

FIGURE 39C (P3)

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP280

186/254

FIGURE 39D

Figure 39. d) Amino acid sequence Comparison of Mutant Preproricin Linker region of Urokinase-Type Plasminogen Activator to Wild Type

Wild type ricin linker:	A chain- S L L I R P V V P N F N -B chain
pAP-280 (uPA) linker:	A chain- K K S P G R V V G G S V-B chain

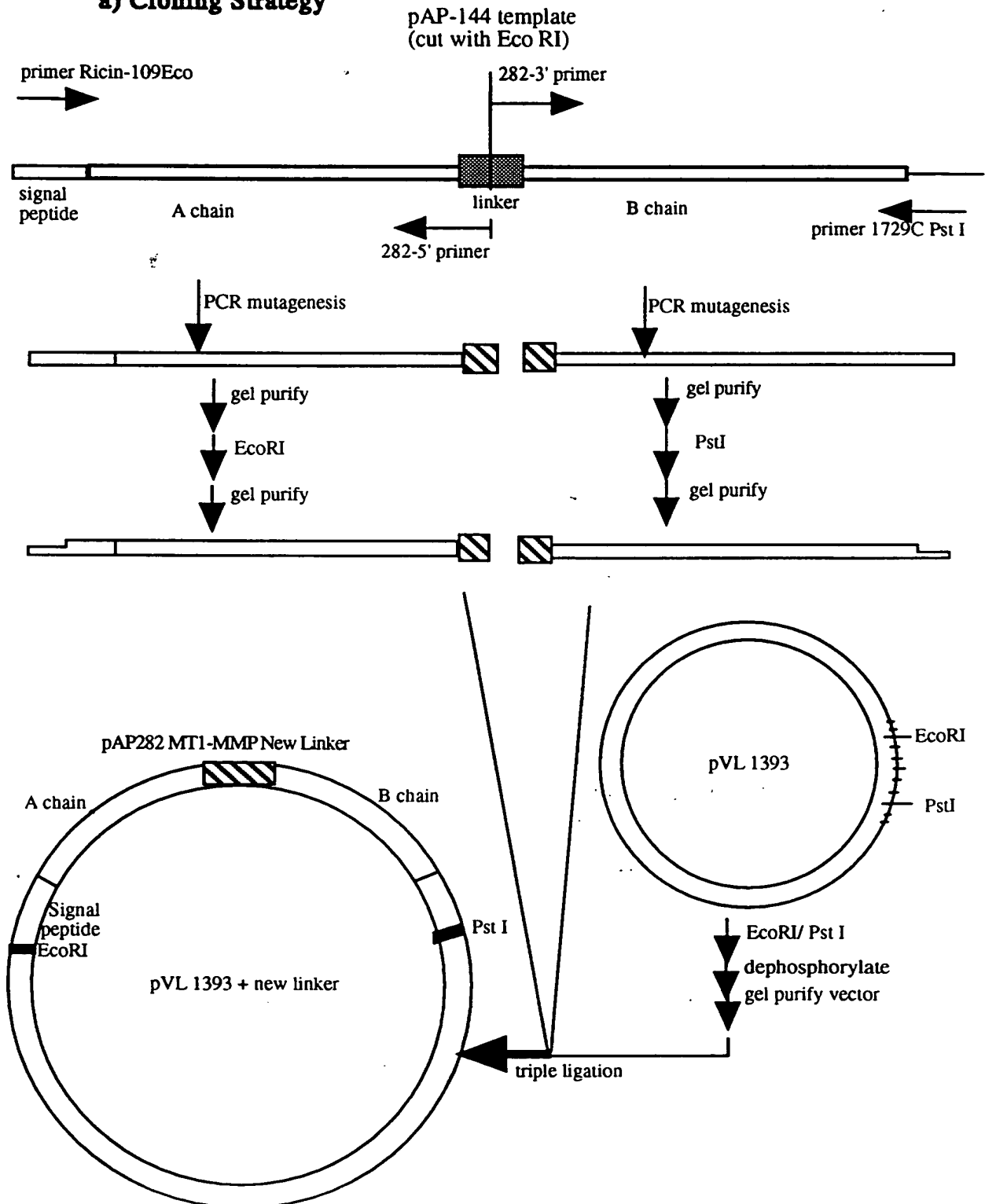
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187/254

FIGURE 40A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



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188/254

FIGURE 40B

Sequence of MT-MMP Linker Region

WT preprocin linker

primer 282-3'

5' - GTCCTGGTATTCTTGGCGCTGATGTTTGT -3'

***** * * * *

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAAATTA-----

* * * * *

3' -AGCAGTGTCAAAGGGGTTCTGAGGATCCC -5'

primer 282-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 282 linker

(MT-MMP variant)

-----CCCCAAGGACTCCTAGGG|GTCCTGGTATTCTTGGC-----
-----GGGGTTCCTGAGGATCCC|CGAGGACCATAAGAACCG-----

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189/254

FIGURE 40C (P1)

Sequence of pAP282 insert

	10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA				
51	GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG				
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTATC				
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
	TCCTATTGTTGTATAAGGGGTTTGTTATGGGTTAATATTTGAAATGGTGT				
151	GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG				
	CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC				
201	TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTTGCCAA				
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251	ACAGAGTTGGTTTGCCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA				
	TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT				
301	AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351	TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA				
	ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT				
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT				
	TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA				
451	CGATATACATTGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC				
	GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTTGAACG				
501	TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTCTTTTATAGCTCAACCCTTACCAGGTGATCTCCTCC				
551	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601	CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG				
	GACCGAGCAAGGAAATATTAACGTTAGGTTTACTAAAGTCTTCGTCGTTT				
651	ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT				

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190/254

FIGURE 40C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTCCCCAAGGACTCCTAGGGGCTCCTGGTATTCTTGGCGC
AGCAGTGTCAAAGGGGTTTCTGAGGATCCCCGAGGACCATAAGAACCGCG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTIONGTTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATAACGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

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191/254

FIGURE 40C (P3)

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP282

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192/254

FIGURE 40D

**Amino acid sequence Comparison of Mutant Preproricin Linker
region of MT-MMP to Wild Type**

Wild type ricin linker:	A chain- S L L I R P V V P N F N -B chain
pAP-282 (MT-MMP) linker:	A chain- P Q G L L G A P G I L G-B chain

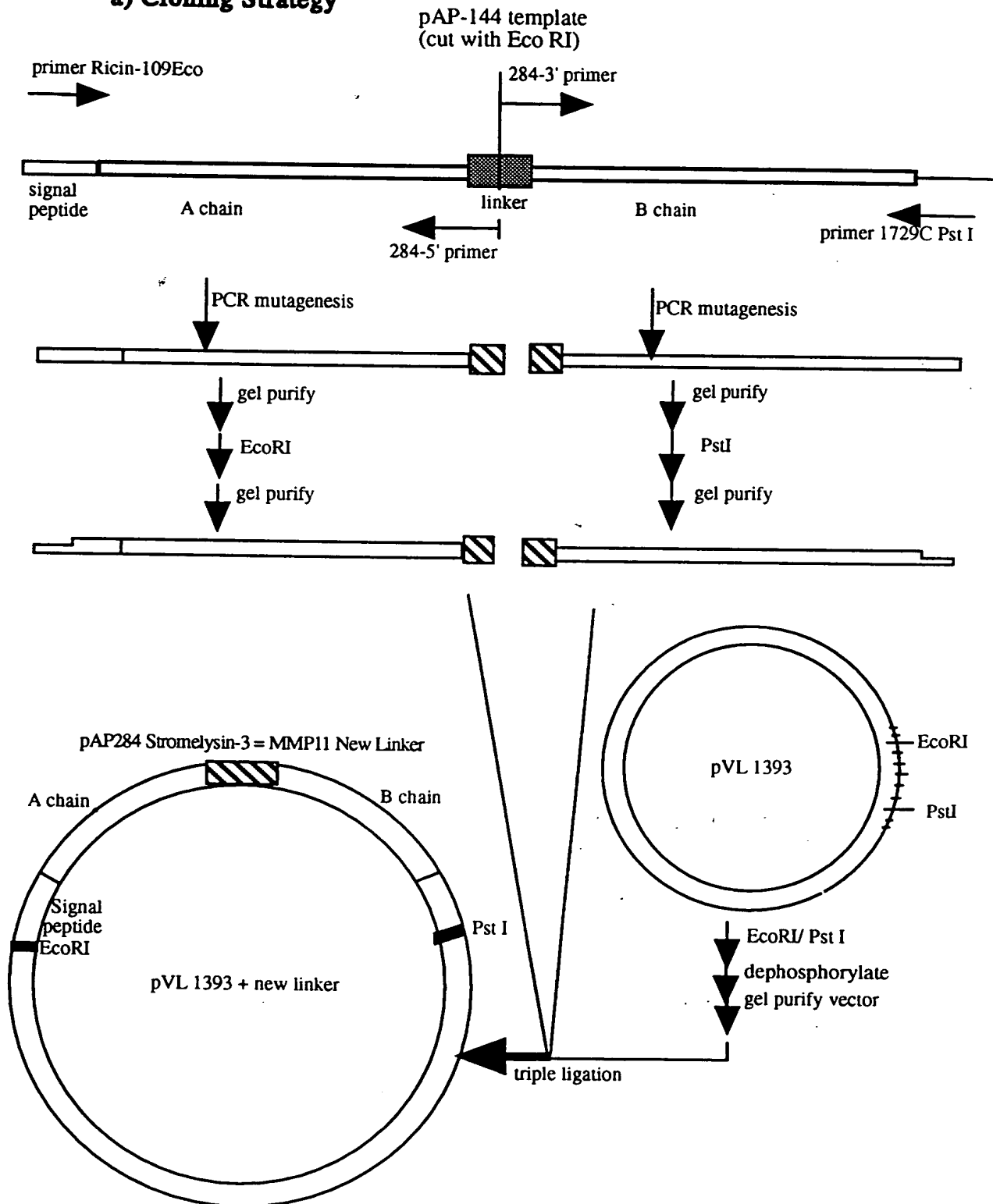
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193/254

FIGURE 41A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



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FIGURE 41B

Sequence of MMP-11 (Stromelysin-3) Linker Region

WT preprocin linker

primer 284-3'

5' - ATGGGAAGAGGCCCATGCTCGTTTAGTTTCATGTCGAAGAGCCTCACACTGCTGATGTTTGTATGGAT-3

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAATTA-----

3'-GGTGGTAGCAGTGTCAAAGTGCCGGGGCTCCCAAATTTCTACCCCTAAATACTTAGACTGCAG -5'
primer 284-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 284 linker

(MMP-11 variant)

---CACGGCCCCGAGGGTTTAAGAGTGGGATTTTATGAATCTGACGTC|ATGGGAAGAGGCCCATGCTCGTTTAGTTTCATGTCGAAGAGCCTCACACT---
---GTGCGGGGGCTCCCAAATTTCTACCCCTAAATACTTAGACTGCAG|TACCCCTTCTCCGGTACGAGCAAAATCAAGTACAGCAACTCGGAGTGTGA---

195/254

FIGURE 41C (P1)

Sequence of pAP284 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGCACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAAGTCAAGTTTTA
451 CGATATACATTGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAAGTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCCTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTC
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

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196/254

FIGURE 41C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTT
AGCAGTGTCAA

Linker Sequence:

CACGGCCCCGAGGGTTTAAGAGTGGGATTTTATGAATCTGACGTCATGGG
GTGCCGGGGCTCCCAAATTCTCACCCCTAAATACTTAGACTGCAGTACCC

AAGAGGCCATGCTCGTTTAGTTCATGTGGAAGAGCCTCACACT
TTCTCCGGTACGAGCAAATCAAGTACAGCAACTCGGAGTGTGA

949 GC
CG

951 TGATGTTTGATGATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGA CTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

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197/254

FIGURE 41C (P3)

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATCCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

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198/254

FIGURE 41D

**Amino acid sequence Comparison of Mutant Preproricin Linker
region of MMP-11 (Stromelysin-3) to Wild Type**

Wild type ricin linker:

A chain- S L L I R P V V P N F N -B chain

pAP-284 (MMP-11) linker:

A chain- H G P E G L R V G F Y E S D V M G R G H A R L V H V E E P H T -B chain

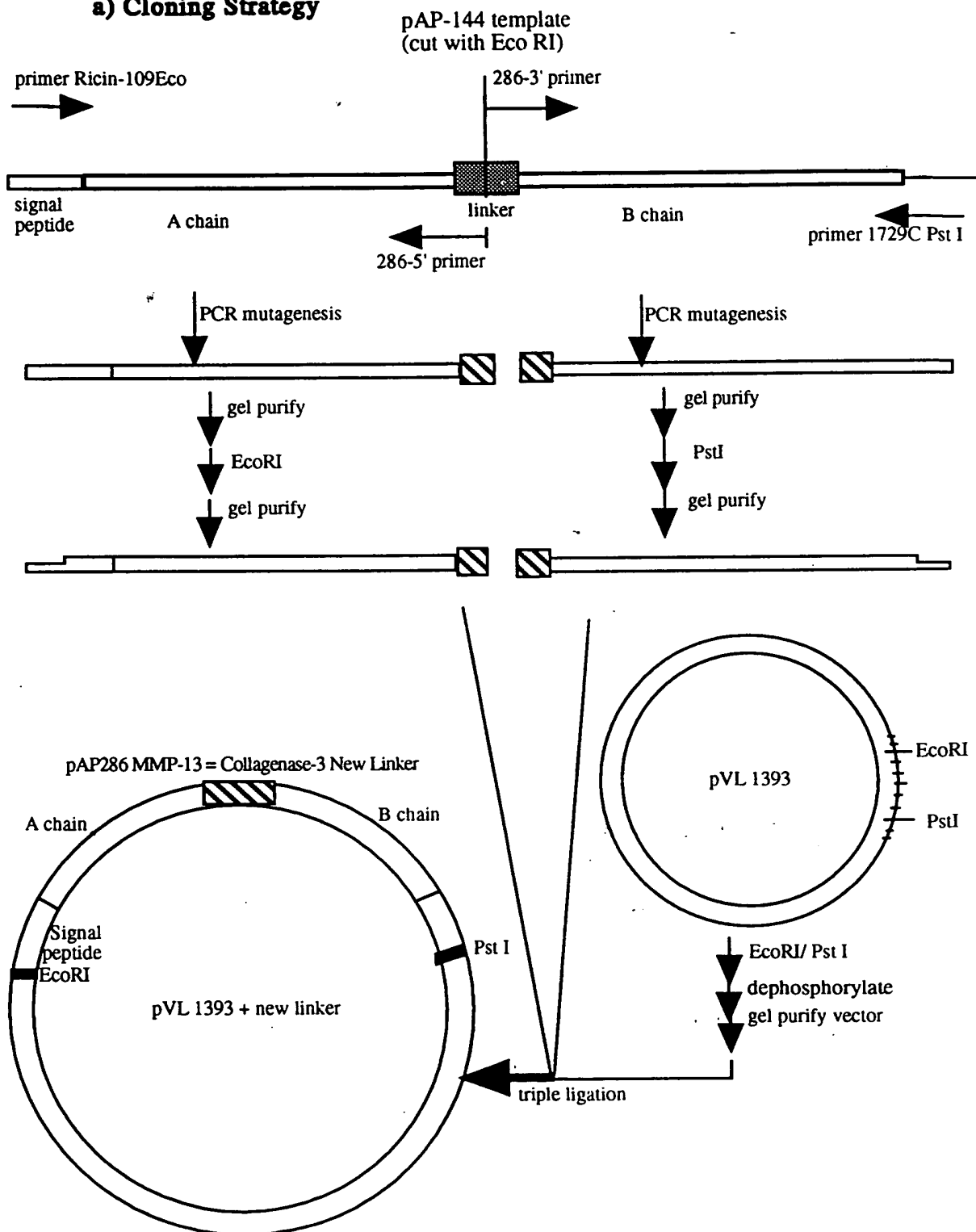
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199/254

FIGURE 42A

PCR Mutagenesis of Preprorincin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



200/254

FIGURE 42B

Sequence of MMP-13 = Collagenase-3 Linker Region

WT preprocin linker

primer 286-3'
5' - GGTCAACGAGGCATTGTCGCTGATGTTTGT -3'
***** * ***** **

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAAATTA-----
***** ***** *

3' -AGCAGTGTCAAACCTGGAGTCCCCGAACGA -5'
primer 286-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 286 linker (MMP-13 variant)

-----GGACCTCAGGGGCTTGCT|GGTCAACGAGGCATTGTC-----
-----CCTGGAGTCCCCGAACGA|CCAGTTGCTCCGTAACAG-----

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201/254

FIGURE 42C (P1)

Sequence of pAP286 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC
101 AGGATAACAACATATTTCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATAACAGTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAATAAAGTTTGA
451 CGATATACATTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACCTGCG
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA

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202/254

FIGURE 42C (P2)

CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTGGACCTCAGGGGCTTGCTGGTCAACGAGGCATTGTGCG
AGCAGTGTCAAACCTGGAGTCCCCGAACGACCAGTTGCTCCGTAACAGCG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTGCAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTGTTACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

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203/254

FIGURE 42C (P3)

GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551 TGTAAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA
1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA
1751 CTCTTGCA GTGTGTGTCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851 TGCAG
ACGTC

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204/254

FIGURE 42D

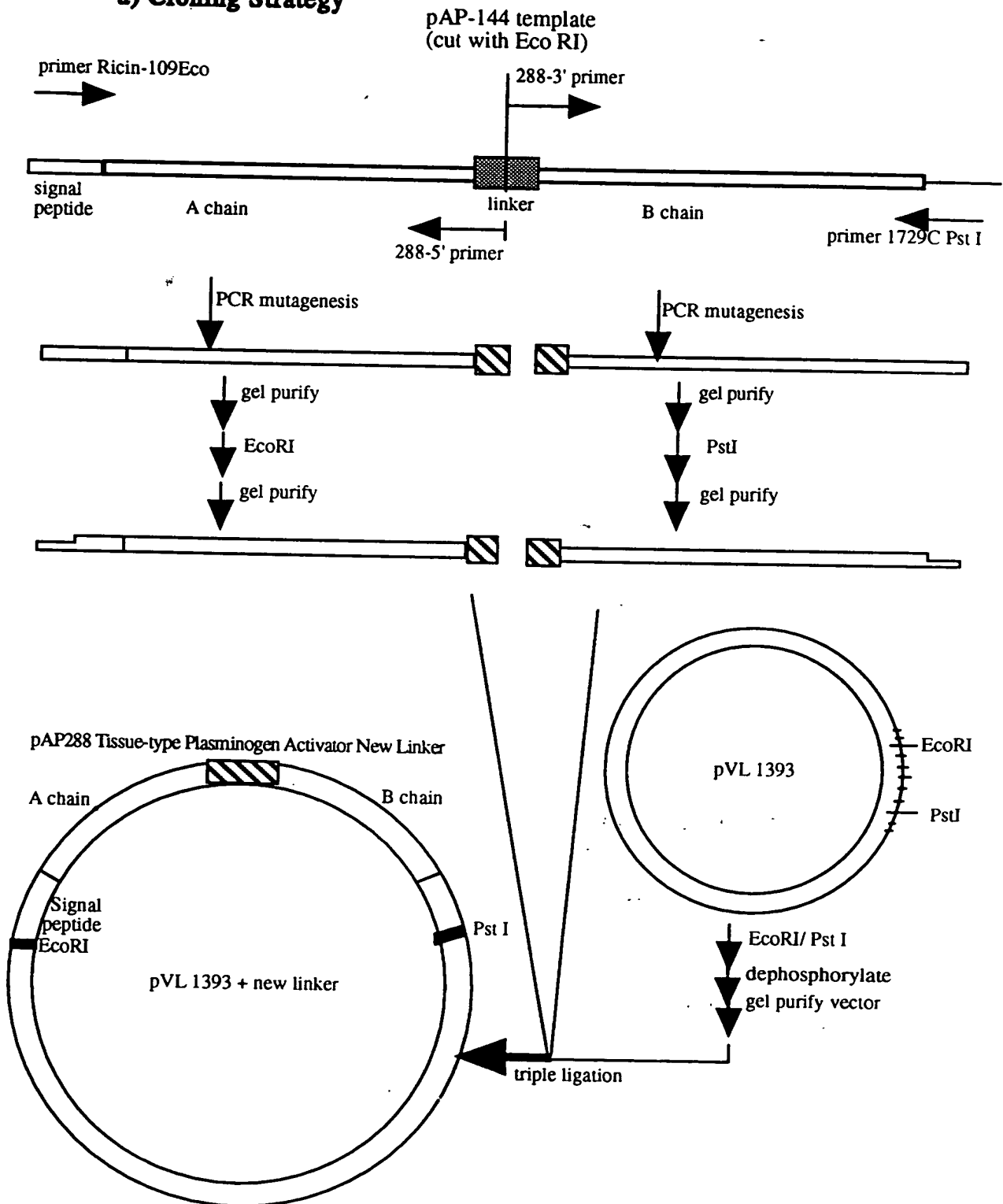
**Amino acid sequence Comparison of Mutant Preproricin Linker
region of MMP-13 (Collagenase-3) to Wild Type**

Wild type ricin linker:	A chain- S L L I R P V V P N F N -B chain
pAP-286 (MMP-13) linker:	A chain- G P Q G L A G Q R G I V -B chain

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FIGURE 43A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy

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206/254

FIGURE 43B

Sequence of Tissue-type Plasminogen Activator (tPA) Linker Region

WT preprocin linker

primer 288-3'
5'- GGTTCGTAAAGCTCTTGAAGCTGATGTTTGT -3'
***** * * * *
-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAAATTA-----

3'-AGCAGTGTCAAACCGCCTAGACCCGTTTCC -5'
primer 288-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 288 linker (tPA variant)

-----GGCGGATCTGGGCAAAGG|GGTCGTAAAGCTCTTGAA-----
-----CCGCCTAGACCCGTTTCC|CCAGCATTTTCGAGAACTT-----

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207/254

FIGURE 43C (P1)

Sequence of pAP288 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACTGAGCTGATGTGAGACATGATATAACAGTGTGCGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAACTATCTGAACTTGTGTAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCCTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA

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208/254

FIGURE 43C (P2)

CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGTTTAAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGTGGT

901 TCGTCAACAGTTTGGCGGATCTGGGCAAAGGGGTCGTAAAGCTCTTGAAGC
AGCAGTGTCAAACCGCCTAGACCCGTTTCCCCAGCATTTCGAGAACTTCG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTACAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTACAAACCATGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

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209/254

FIGURE 43C (P3)

GTTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA
1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701 TGGTGAÇCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA
1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP288

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Amino acid sequence Comparison of Mutant Preproricin Linker region of Tissue-type Plasminogen Activator (tPA) to Wild Type

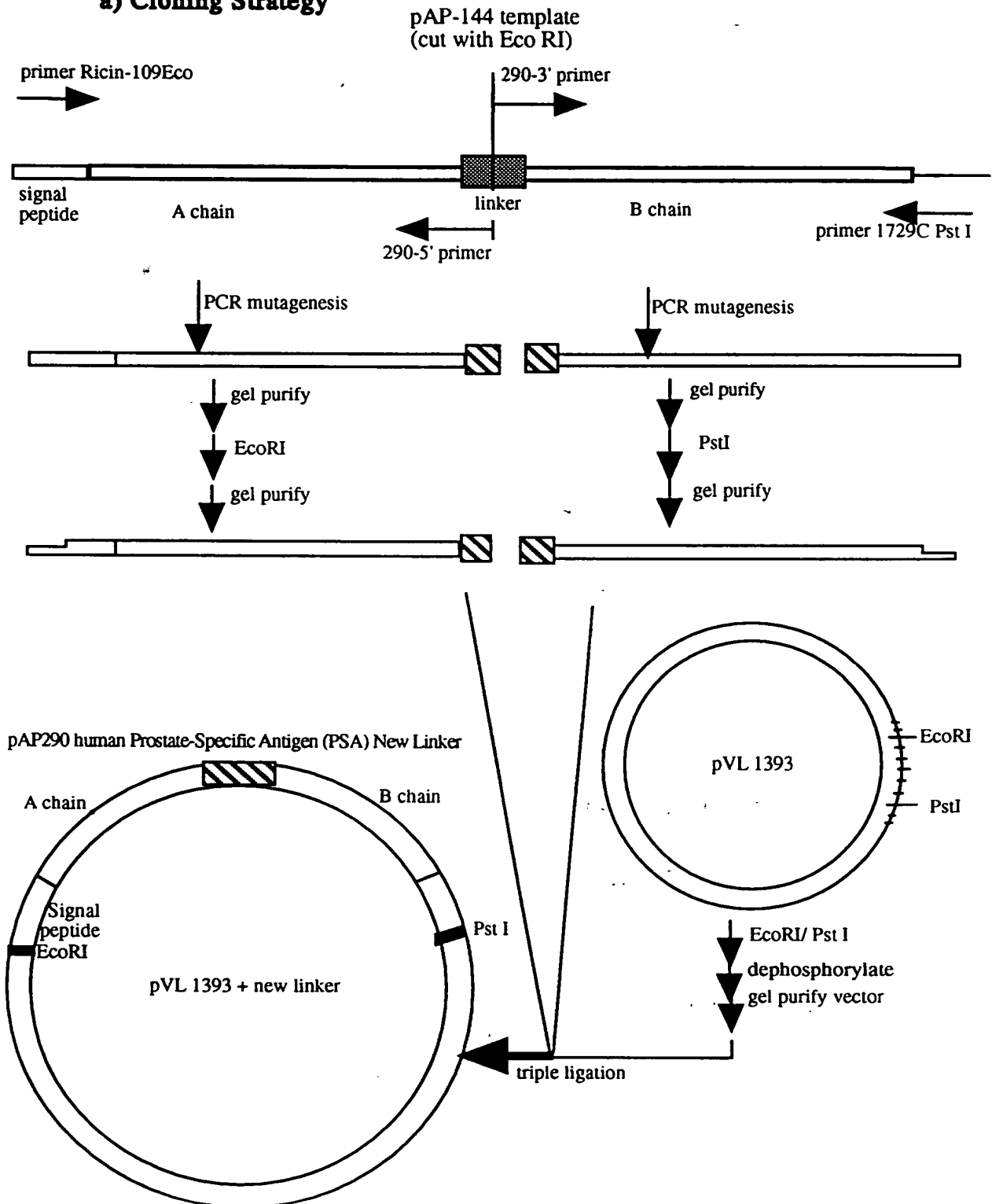
Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain
pAP-288 (tPA) linker: A chain- G G S G Q R G R K A L E-B chain

211/254

FIGURE 44A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy



212/254

FIGURE 44B

Sequence of human Prostate-Specific Antigen (PSA) Linker Region

WT preprocin linker

primer 290-3'

5' - TCTTCCGATATTTTAAATGCTGATGTTTGT -3'

***** *

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAAATTA-----

***** *

3' -AGCAGTGTCAAAGAAACAGTCGAGAAGAG -5'

primer 290-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 290 linker

(PSA variant)

-----TCTTTGTCAGCTCTTCTC|TCTTCCGATATTTTAAAT-----
-----AGAAACAGTCGAGAAGAG|AGAAGGCTATAAAAATTA-----

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213/254

FIGURE 44C (P1)

Sequence of pAP290 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTAAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGCG
CGCCCACTGGTGACACGTTTCGATGTGTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGAATAAAGTTTAA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAACCACTTAATACTATCTGAACTTGTGTAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA

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214/254

FIGURE 44C (P2)

CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTTTGTCAGCTCTTCTCTCTTCCGATATTTTTAATGC
AGCAGTGTCAAAGAAACAGTCGAGAAGAGAGAAGGCTATAAAAATTACG

951 TGATGTGTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTGCAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTACAGTTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTTACAAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATAACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTACATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

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215/254

FIGURE 44C (P3)

GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT

1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP290

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216/254

FIGURE 44D

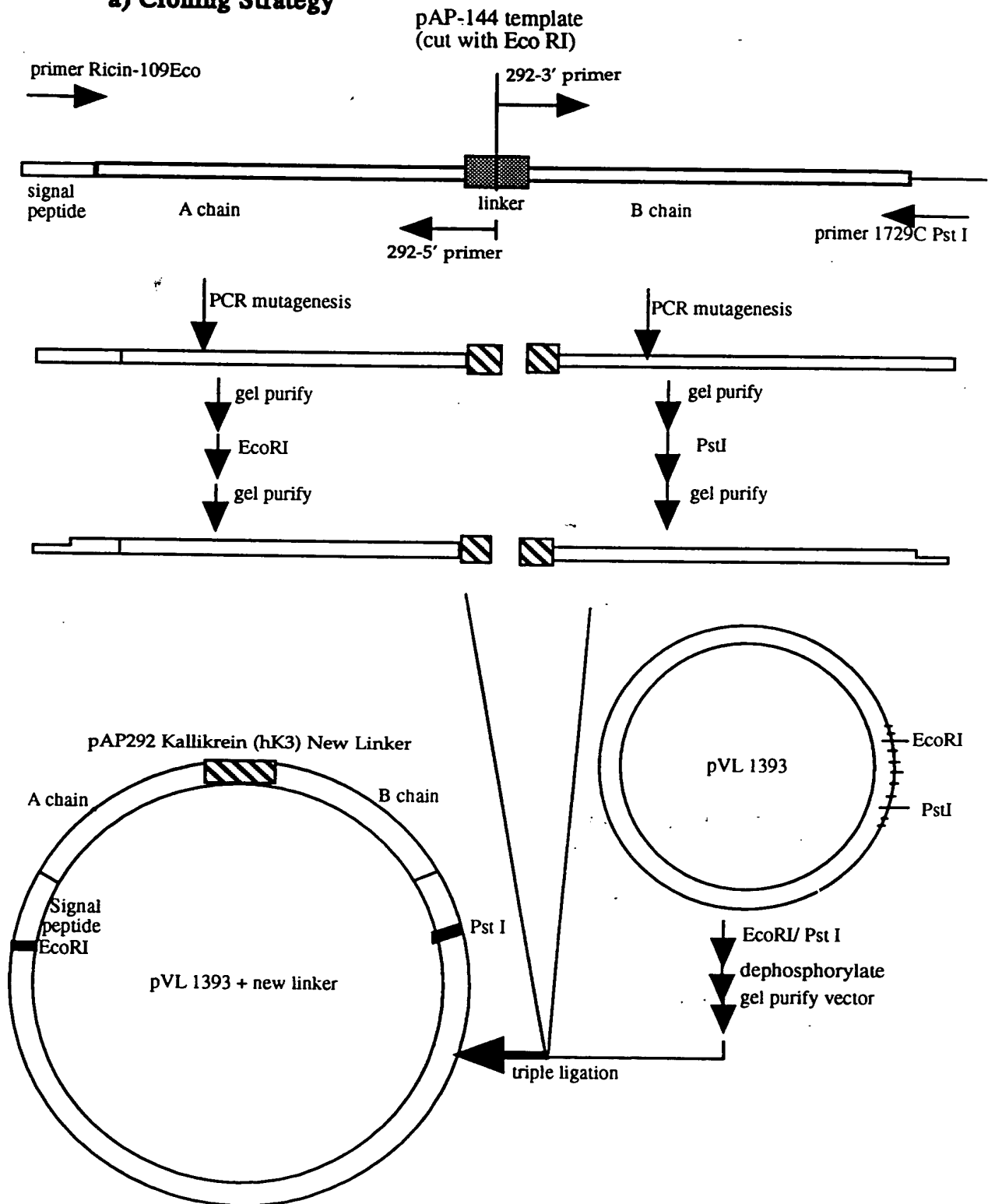
**Amino acid sequence Comparison of Mutant Preproricin Linker
region of human Prostate-Specific Antigen (PSA) to Wild Type**

Wild type ricin linker:	A chain- S L L I R P V V P N F N -B chain
pAP-290 (PSA) linker:	A chain- S L S A L L S S D I F N -B chain

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FIGURE 45A

PCR Mutagenesis of Preproricein Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy

218/254

FIGURE 45B

Sequence of Kallikrein (hK3) Linker Region

WT preprocin linker

primer 292-3'

5' - ATTATCGGTGGCTTTAATGCTGATGTTTGT -3'

* ** *****

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAATTA-----

* * *****

3' - AGCAGTGTCAAAGAAACGGATCTAAATTT -5'

primer 292-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 292 linker

(Kallikrein variant)

-----TCTTTGCCTAGATTTAAA|ATTATCGGTGGCTTTAAT-----
-----AGAAACGGATCTAAATTT|TAATAGCCACCGAAATTA-----

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219/254

FIGURE 45C (P1)

Sequence of pAP292 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451 CGATATACATTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
TATAGAGTTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTTCAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA

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220/254

FIGURE 45C (P2)

CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTTTGCCTAGATTTAAAATTATCGGTGGCTTTAATGC
AGCAGTGTCAAAGAAACGGATCTAAATTTAATAGCCACCGAAATTACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAAC TACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTT CAGATTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGT TACAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTGTTTTATCACCTGTT CATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGT CACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

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221/254

FIGURE 45C (P3)

GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA
1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701 TGGTGACCCAAACCAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA
1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
1801 GGACATTGTAAATTTTGTAACCTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP292

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222/254

FIGURE 45D

Amino acid sequence Comparison of Mutant Preproricin Linker
region of Kallikrein (hK3) to Wild Type

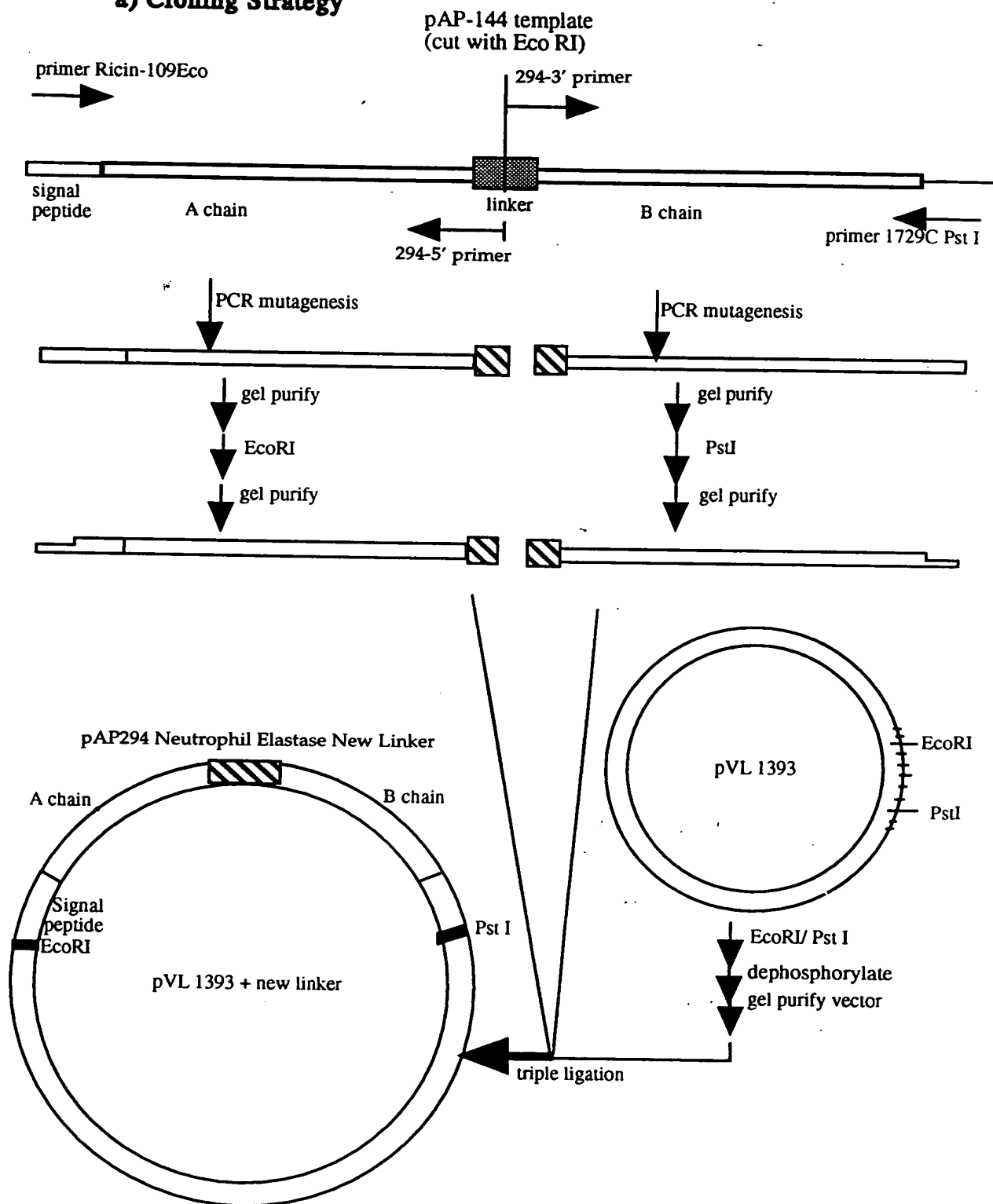
Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-292 (hK3) linker: A chain- S L P R F K I I G G F N -B chain

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FIGURE 46A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy

224/254

FIGURE 46B

Sequence of Neutrophil Elastase Linker Region

WT preprocin linker

primer 294-3'

5'- GTTCCTGGTAATTTTAATGCTGATGTTTGT -3'

** *****

-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAATTA-----

*** *** *

3'-AGCAGTGTCAAAAGAAACGAACCGTAACGA -5'

primer 294-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 294 linker

(Neutrophil elastase variant)

-----TCTTTGCTTGGCATTGCT|GTTCCTGGTAATTTTAAT-----
-----AGAAACGAACCGTAACGA|CAAGGACCATTAAATTA-----

095151.04400

225/254

FIGURE 46C (P1)

Sequence of pAP294 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTGCGGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351 TGTGGTTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGCACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACCTGC
GCTATATGTAAGCGGAAACCACCATTAAATACTATCTGAACTTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA

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FIGURE 46C (P2)

CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTTTCTTTGCTTGGCATTGCTGTTCTGGTAATTTTAATGC
AGCAGTGTCAAAGAAACGAACCGTAACGACAAGGACCATTAAATACG

951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACCTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAAGTTATGTCTACGTTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT

1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

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227/254

FIGURE 46C (P3)

GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551 TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA
1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGT TAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701 TGGTGACCCAAACCAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA
1751 CTCTTGCA GTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
1801 GGACATTGTAAATTTTGTAAGTAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP294

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228/254

FIGURE 46D

**Amino acid sequence Comparison of Mutant Preproricin Linker
region of Neutrophil elastase to Wild Type**

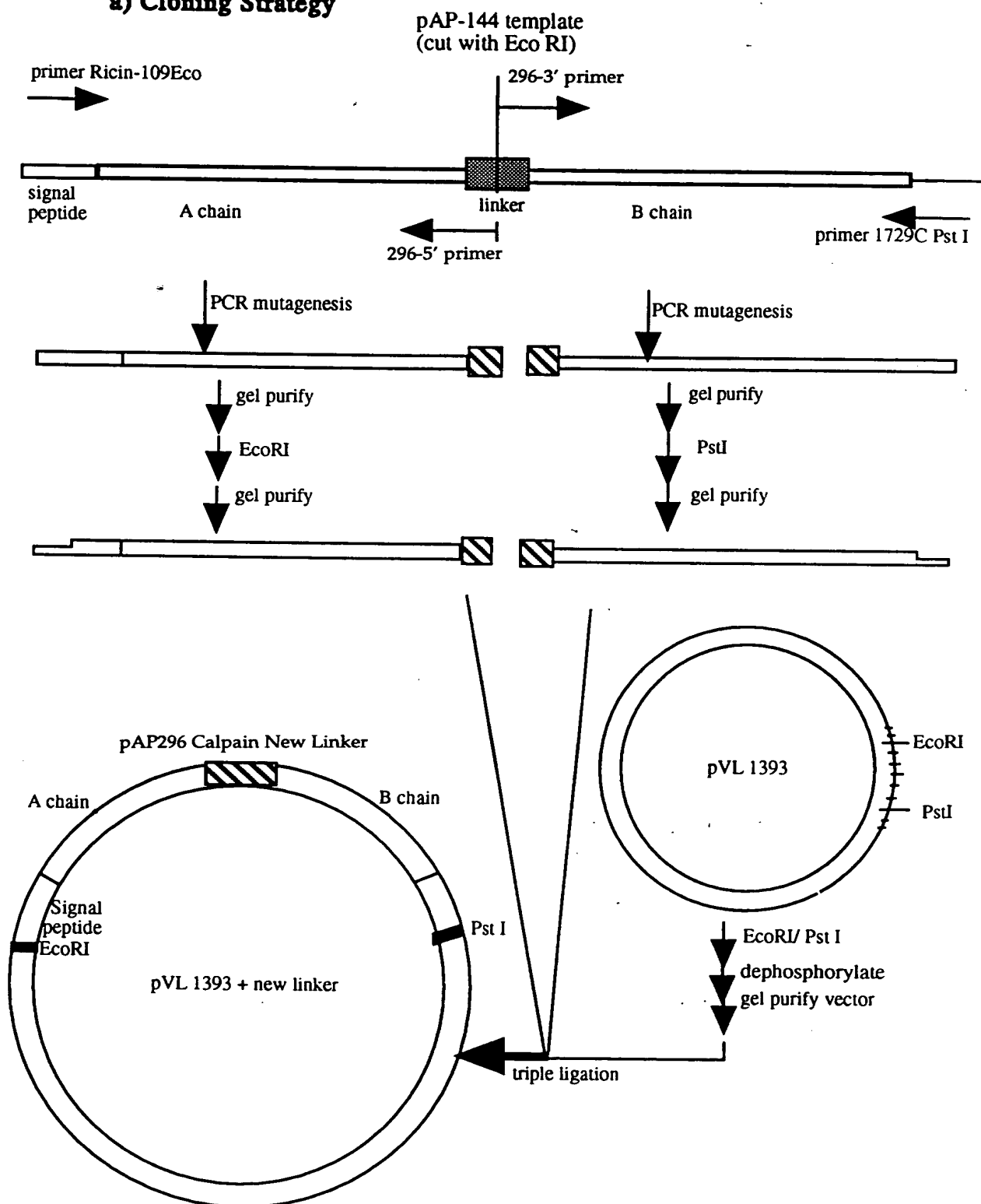
Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

**pAP-294 (Neutrophil elastase) linker:
 A chain- S L L G I A V P G N F N -B chain**

00440" TESTS60

FIGURE 47A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393

a) Cloning Strategy

0047T40" TSTTS60

230/254

FIGURE 47B

Sequence of Calpain Linker Region

WT preprocin linker

primer 296-3'

5'- ACTCCTAGAACCCCCCAGCTGATGTTTGT -3'

```
-----TCTTTGCTTATAAGGCCA|GTGGTACCAAATTTTAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTTAAAATTA-----
          *   * * * *
          *   * * * *
```

3'-AGCAGTGTCAAAAAAAGTTTTATAACAA -5'

primer 296-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 296 linker

(Calpain variant)

```
-----TTTTTCAAAAATATTGTT|ACTCCTAGAACCCCCCA-----
-----AAAAAGTTTTTATAACAA|TGAGGATCTTGGGGGGGT-----
```

005454 " 044400

231/254

FIGURE 47C (P1)

Sequence of pAP296 insert

10 20 30 40 50
| | | | |
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51 GGCAACATGGCTTTGTTTTGGATCCACCTCAGGGTGGTCTTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
TCCTATTGTTGTATAAGGGGTTTGTATGGGTTAATATTTGAAATGGTGT
151 GCGGGTGCCACTGTGCAAAGCTACACAACTTTATCAGAGCTGTTTCGCGG
CGCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201 TCGTTTAACAACCTGGAGCTGATGTGAGACATGATATACCAGTGTTGCCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT
251 ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301 AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTACGTAT
351 TGTGGTCCGCTACCGTGCTGGAAATAGCGCATATTTCTTTCATCCTGACA
ACACCAGCCGATGGCAGACCTTTATCGCGTATAAAGAAAGTAGGACTGT
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451 CGATATACATTTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601 CTGGCTCGTTCCTTTATAATTTGCATCCAAATGATTTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTT
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA

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232/254

FIGURE 47C (P2)

CTAGACGTGGTCTAGGATCGCATTAAATGTGAACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
AGTTGACGTTTCTGCATTACCAAGGTTTAAAGTCACACATGCTACACTCAT
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
901 TCGTCACAGTTTTTTTTTCAAAAATATTGTTACTCCTAGAACCCCCCAGC
AGCAGTGTCAAAAAAAGTTTTTATAACAATGAGGATCTTGGGGGGGTGCG
951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
CAGATACACAACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
GTCAACACCGGTACGTTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
CCATGTGACGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251 CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351 AATAATACACAACCTTTTGTACCAACCATTTGTTGGGCTATATGGTCTGTG
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
GAACGTTTCGTTTATCACCTGTTTCATACCTATCTCCTGACATCGTCACTTT
1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

0953451 044400

233/254

FIGURE 47C (P3)

GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551 TGTAAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA
1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGT TAGAT
AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701 TGGTGACCCAAACCAATATGGTTACCATTATTTTGATAGACAGATTACT
ACCACTGGGTTTGGTTTATACCAATGGTAATAAACTATCTGTCTAATGA
1751 CTCTTGCA GTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAA
GAGAACGTCACACACACAGGACGGTACTTTTATCTACCGAATTTATTTTT
1801 GGACATTGTAAATTTTGTA ACTGAAAGGACAGCAAGTTATATCGAATTCC
CCTGTAACATTTAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851 TGCAG
ACGTC

Total number of bases is: 1855.

Sequence name: PAP296

09551151.041400

234/254

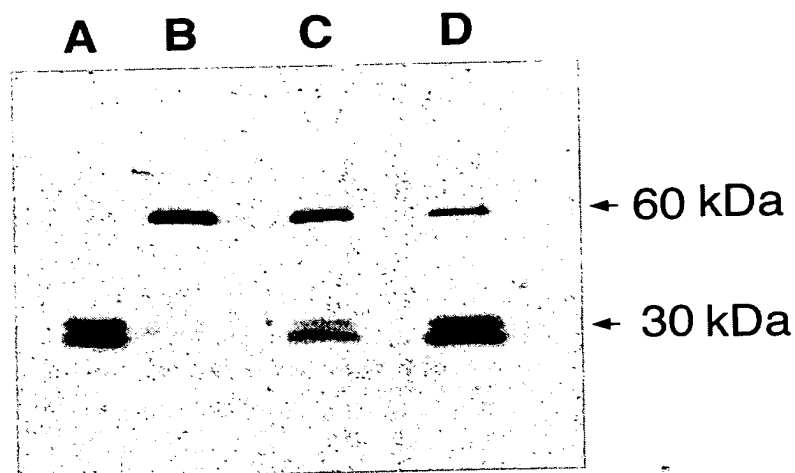
FIGURE 47D

Amino acid sequence Comparison of Mutant Preproricin Linker
region of Calpain to Wild Type

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-296 (Calpain) linker: A chain- F F K N I V T P R T P P -B chain

0955151 041400

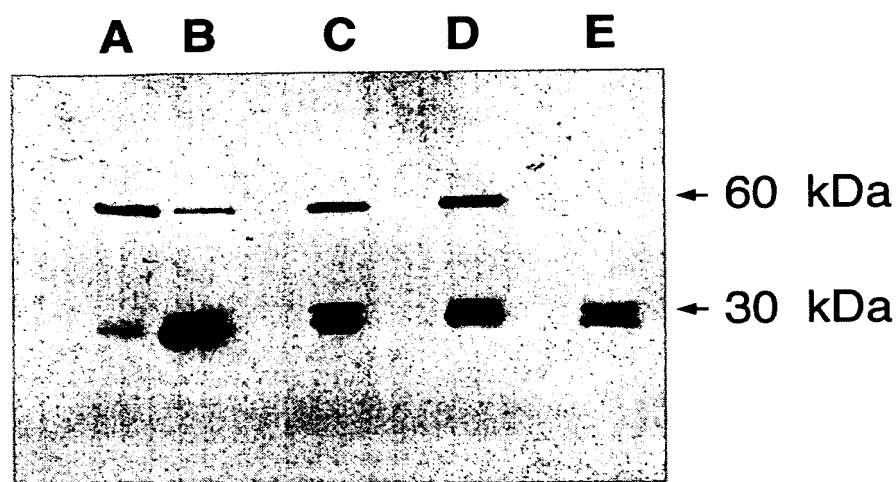
FIGURE 48**Cleavage of pAP 214 by Cathepsin B**

A. Ricin standard

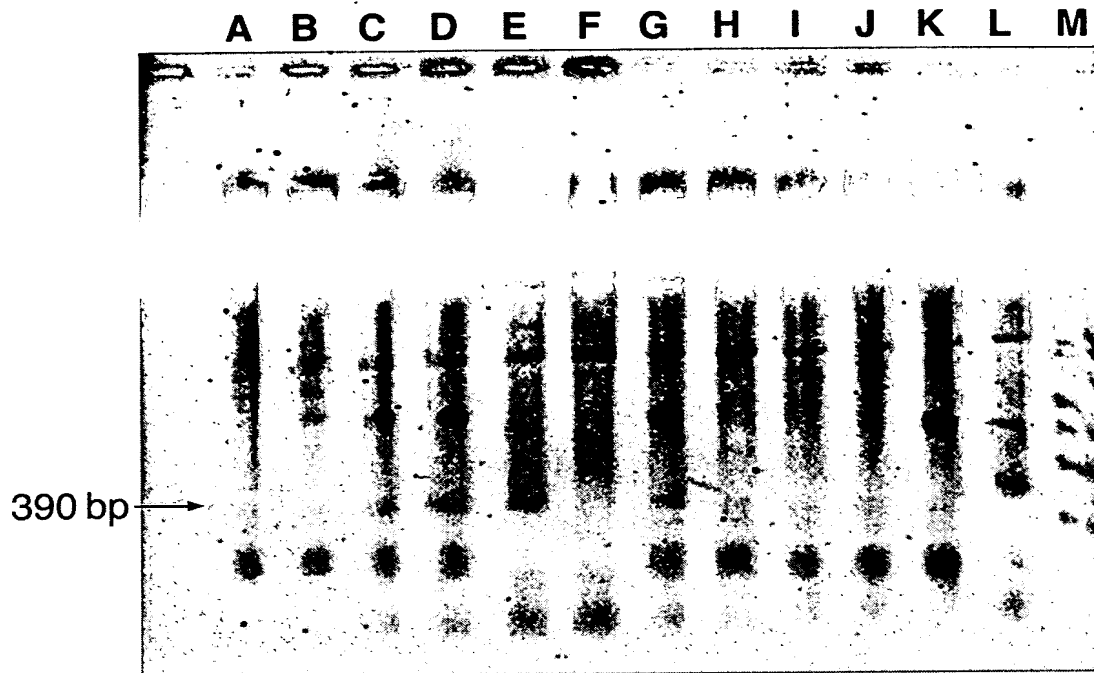
B. pAP 214

C. pAP 214 digested with 100 ng of Cathepsin B (18 hours)

D. pAP 214 digested with 618 ng of Cathepsin B (18 hours)

FIGURE 49**Cleavage of pAP 220 with MMP-9**

- A.** pAP 220
- B.** pAP 220 digested with 200 ng of MMP-9 (16 hrs)
- C.** pAP 220 digested with 20 ng of MMP-9 (16hrs)
- D.** pAP 220 digested with 20 ng of MMP-9 (2hrs)

FIGURE 50**Activation of pAP 214**

- A.** 41.7 pg of pAP 214 digested with Cathepsin B
- B.** 291 pg of pAP 214 digested with Cathepsin B
- C.** 2.0 ng of pAP 214 digested with Cathepsin B
- D.** 14.2 ng of pAP 214 digested with Cathepsin B
- E.** 100 ng of pAP 214 digested with Cathepsin B
- F.** Negative control
- G.** Ricin A chain
- H.** 41.7 pg of pAP 214 variant
- I.** 291 pg of pAP 214 variant
- J.** 2.0 ng of pAP 214 variant
- K.** 14.2 ng of pAP 214 variant
- L.** 100ng of pAP 214 variant
- M.** RNA ladder

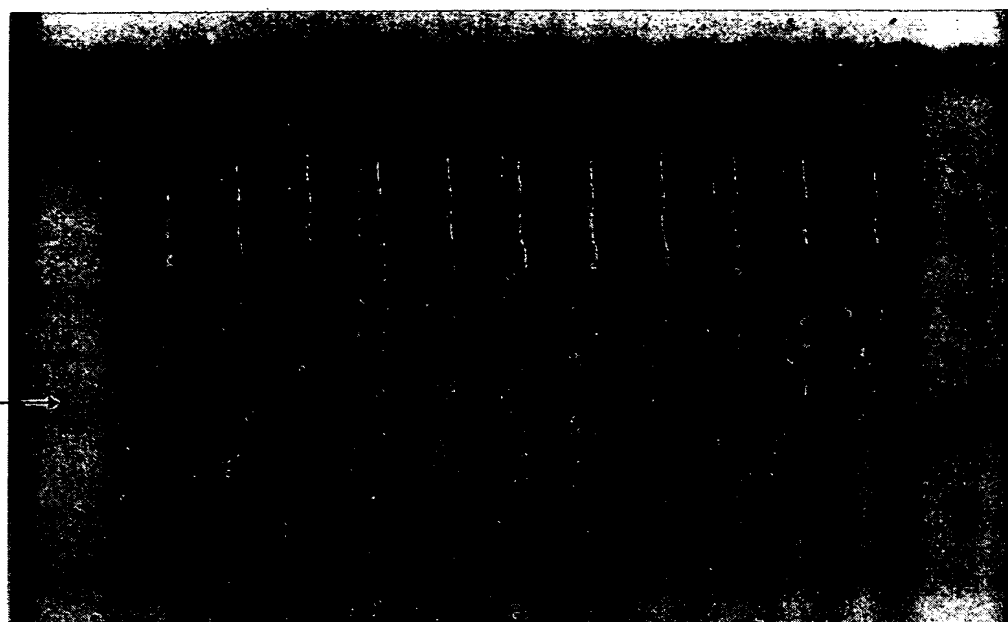
238/254

FIGURE 51

Activation of pAP 220

A B C D E F G H I J K L M

390 bp →



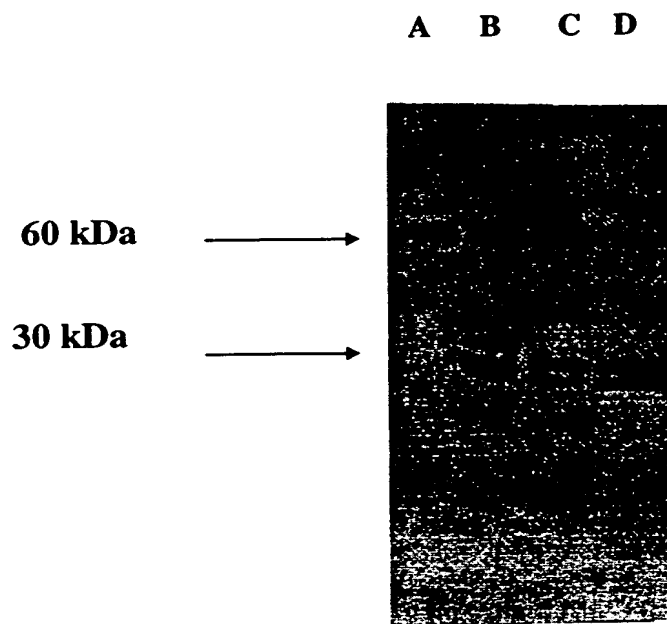
- A. 48.5 pg of pAP 220 variant
- B. 291 pg of pAP 220 variant
- C. 2.0 ng of pAP 220 variant
- D. 14.3 ng of pAP 220 variant
- E. 100 ng of pAP 220 variant
- F. Ricin A chain
- G. Negative Control
- H. 48.5 pg of pAP 220 variant digested with MMP-9
- I. 291 pg of pAP 220 variant digested with MMP-9
- J. 2.0 ng of pAP 220 variant digested with MMP-9
- K. 14.3 ng of pAP 220 variant digested with MMP-9
- L. 100 ng of pAP 220 variant digested with MMP-9
- M. RNA ladder

00440" F5F5560

239/254

FIGURE 52

Cleavage of pAP-248 Protein by The Human Cytomegalovirus (HCMV) protease



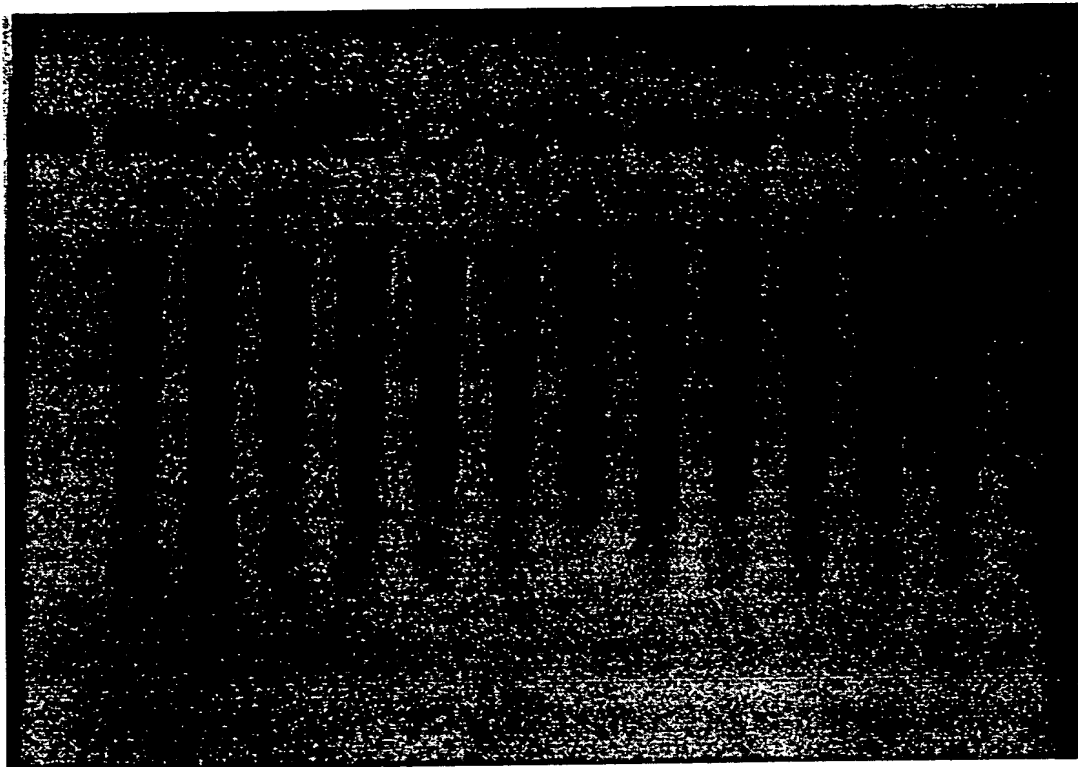
- A. pAP-248 (0.279 μ g)
B. pAP-248 protein (0.279 μ g) digested with 0.25 μ g of the HCMV protease
C. Ricin standard (20 ng)
D. Ricin standard (40 ng)

240/254

FIGURE 53

Activation of pAP-248 Protein

A B C D E F G H I J K L M

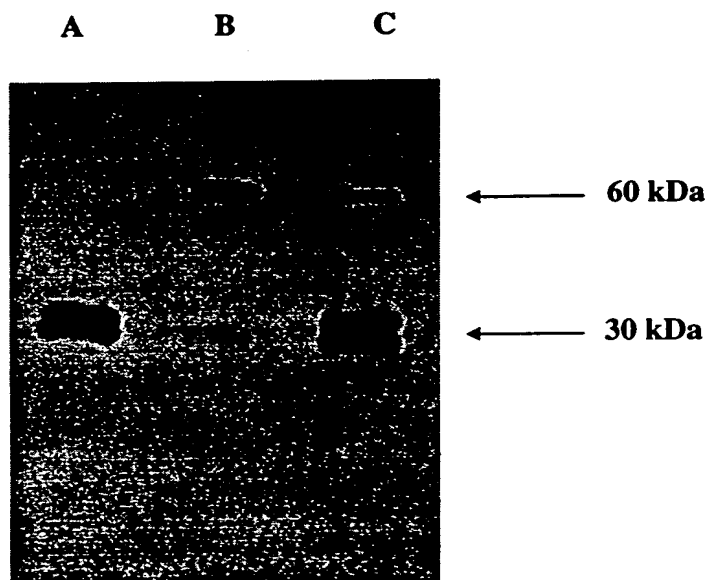


- A. 90 ng of pAP-248 variant**
- B. 12.8 ng of pAP-248 variant**
- C. 1.8 ng of pAP-248 variant**
- D. 260 pg pAP-248 variant**
- E. 37 pg of pAP-248 variant**
- F. Negative control**
- G. Ricin A chain**
- H. 37 pg of pAP-248 digested with HCMV protease**
- I. 260 pg of pAP-248 digested with HCMV protease**
- J. 1.8 ng of pAP-248 digested with HCMV protease**
- K. 12.8 ng of pAP-248 digested with HCMV protease**
- L. 90 ng of pAP-248 digested with HCMV protease**
- M. RNA ladder**

241/254

FIGURE 54

Cleavage of pAP-256 protein by The Hepatitis A Virus 3C (HAV 3C) Protease



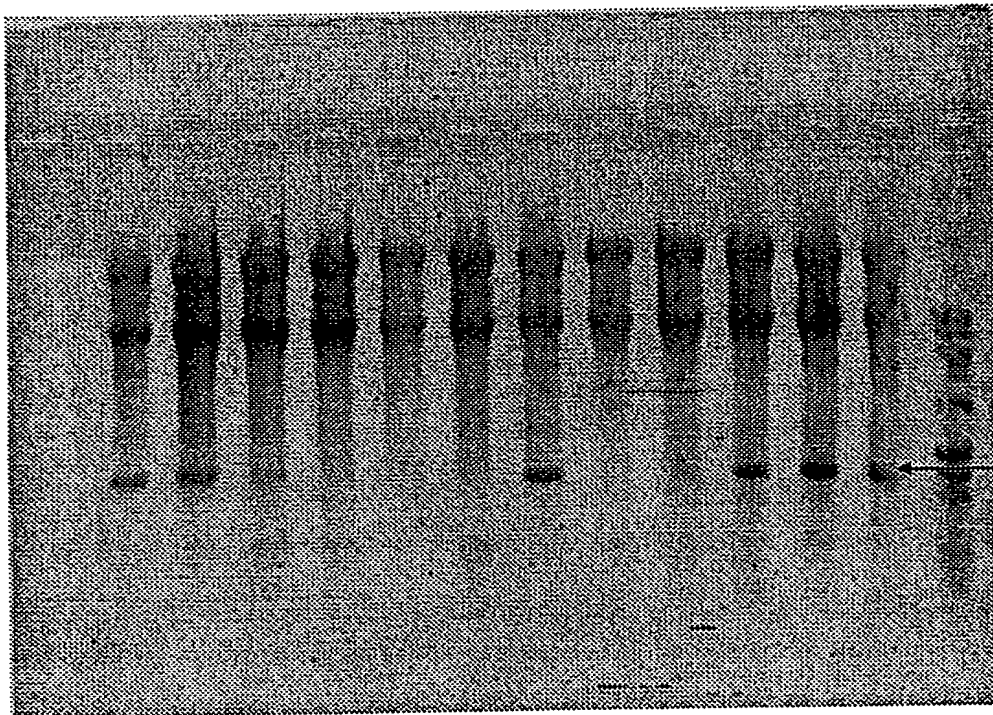
- A. Ricin standard (0.250 ug)
B. pAP-256 protein (0.378 ug)
C. pAP-256 protein digested (0.302 ug) with 1.25 μ g of the HAV 3C protease

242/254

FIGURE 55

Activation of pAP-256 Protein

A B C D E F G H I J K L M

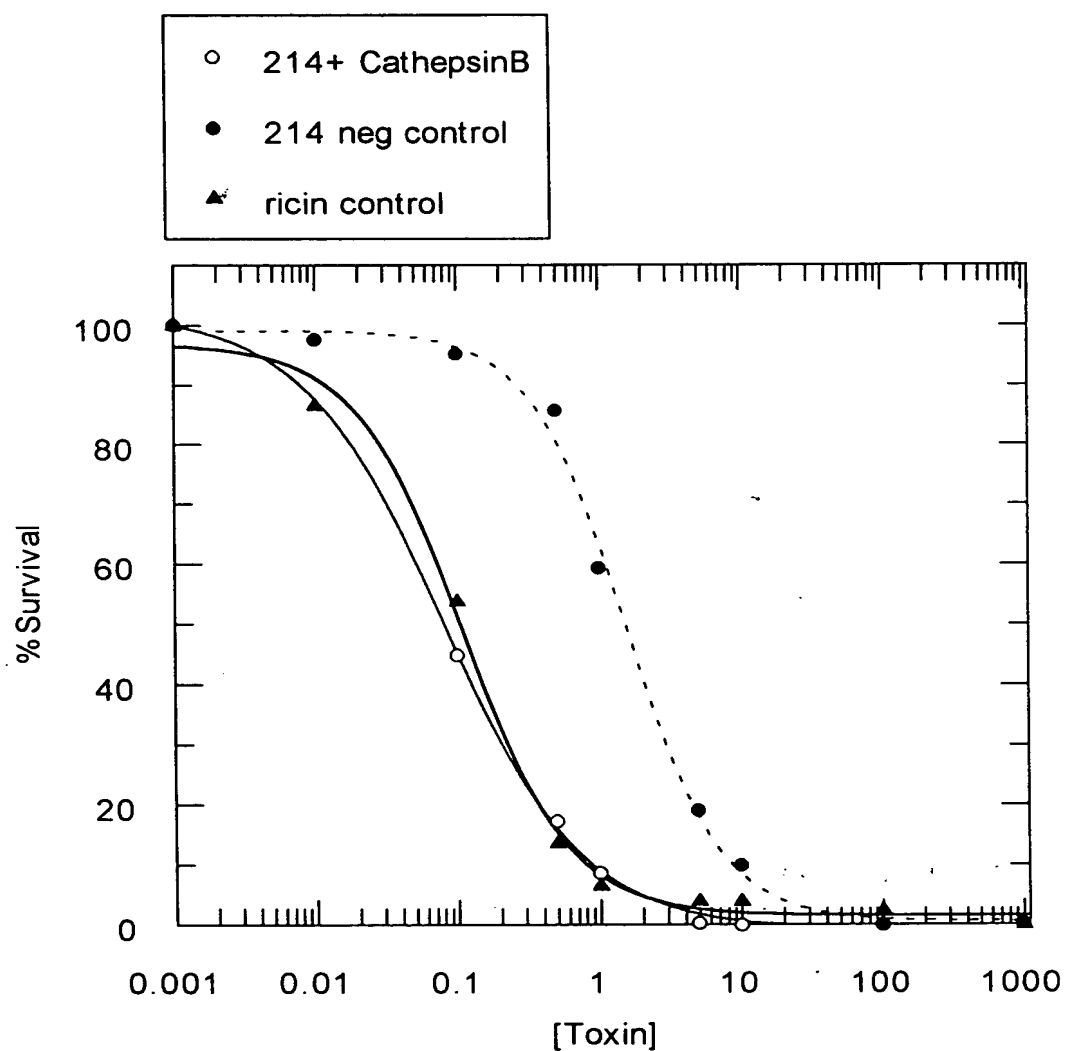


390 b
fragment

- A. 100 ng of pAP-256 variant**
- B. 14.2 ng of pAP-256 variant**
- C. 2.0 ng of pAP-256 variant**
- D. 291 pg of pAP-256 variant**
- E. 41.7 pg of pAP-256 variant**
- F. Negative control**
- G. Ricin A chain**
- H. 41.7 pg of pAP-256 digested with HAV 3C protease**
- I. 291 pg of pAP-256 digested with HAV 3C protease**
- J. 2.0 ng of pAP-256 digested with HAV 3C protease**
- K. 14.2 ng of pAP-256 digested with HAV 3C protease**
- L. 100 ng of pAP-256 digested with HAV 3C protease**
- M. RNA ladder**

FIGURE 56

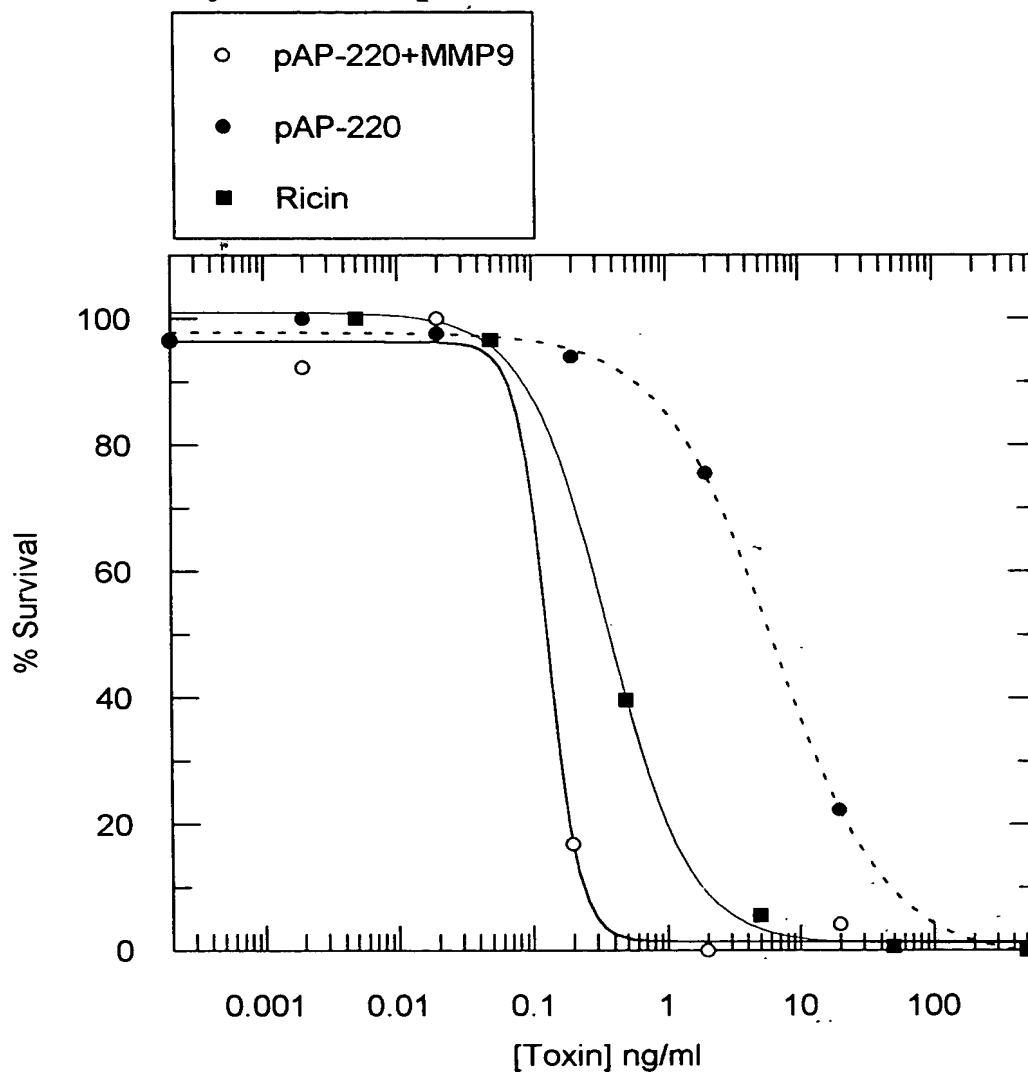
**Cytotoxicity of Digested and Undigested
pAP 214 with Cathepsin B to COS-1 Cells**



	Ricin	pAP 214	pAP 214 + Cathepsin B
IC ₅₀ (ng/ml)	0.11	1.9	0.078
Relative Toxicity	1X	17X	0.7X

FIGURE 57

Cytotoxicity of pAP220 Digested with MMP-9 Compared to Freshly Thawed pAP220 and Ricin on COS-1 Cells

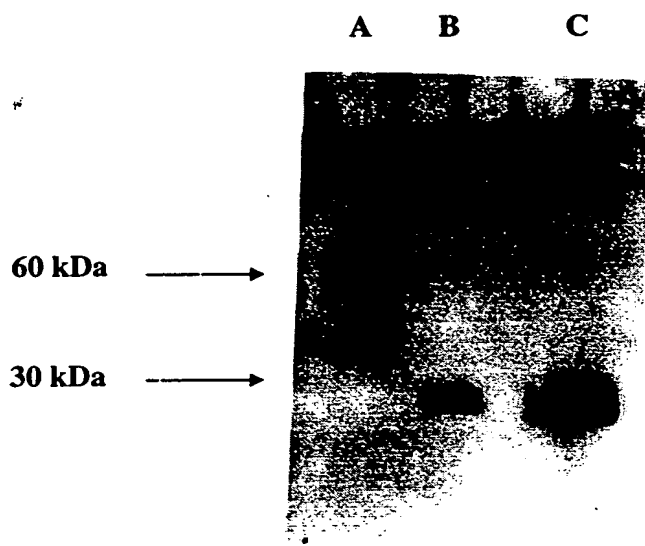


	Ricin	pAP 220	pAP 220 + MMP-9
IC ₅₀ (ng/ml)	0.31	6.7	0.13
Relative Toxicity	1X	22X	0.4X

245/254

FIGURE 58

Cleavage of pAP-270 protein by The Matrix Metalloproteinase 2 (MMP-2)



A. pAP-270 (0.120 µg) undigested

B. pAP-270 (0.120 µg) digested with 0.250 µg MMP-2

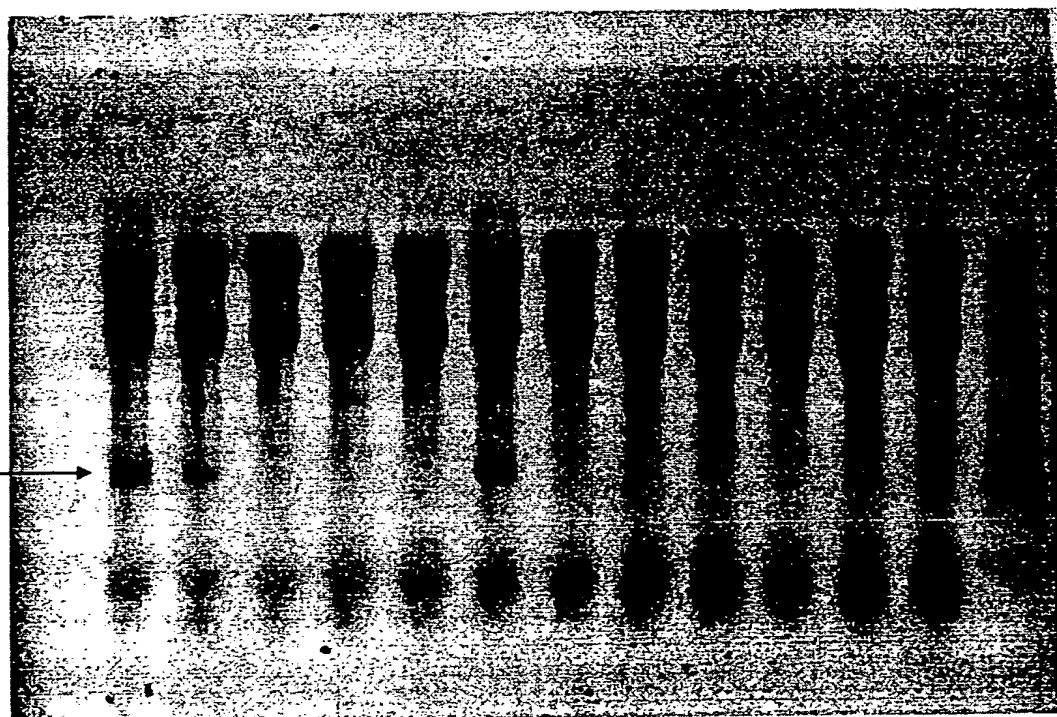
C. Ricin Standard (0.05 µg)

FIGURE 59

Activation of pAP-270 protein

A B C D E F G H I J K L M

390 b
fragment

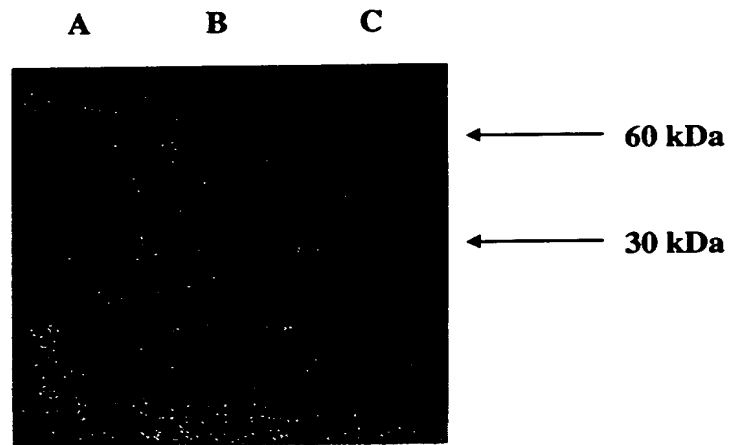


- A. 100 ng of digested pAP-270
- B. 14.2 ng of digested pAP-270
- C. 2.0 ng of digested pAP-270
- D. 290 pg of digested pAP-270
- E. 46 ng of digested pAP-270
- F. Ricin A chain
- G. Negative control
- H. 46 pg of pAP-270
- I. 290 pg of pAP-270
- J. 2.0 ng of pAP-270
- K. 14.2 ng of pAP-270
- L. 100 ng of pAP-270

247/254

FIGURE 60

Cleavage of pAP-288 protein by Plasminogen Tissue Activator (t-PA)



A. Ricin Standard (0.05 μ g)

B. pAP-288 (0.66 μ g) undigested

C. pAP-288 (0.60 μ g) digested with 0.18 μ g of t-PA protease

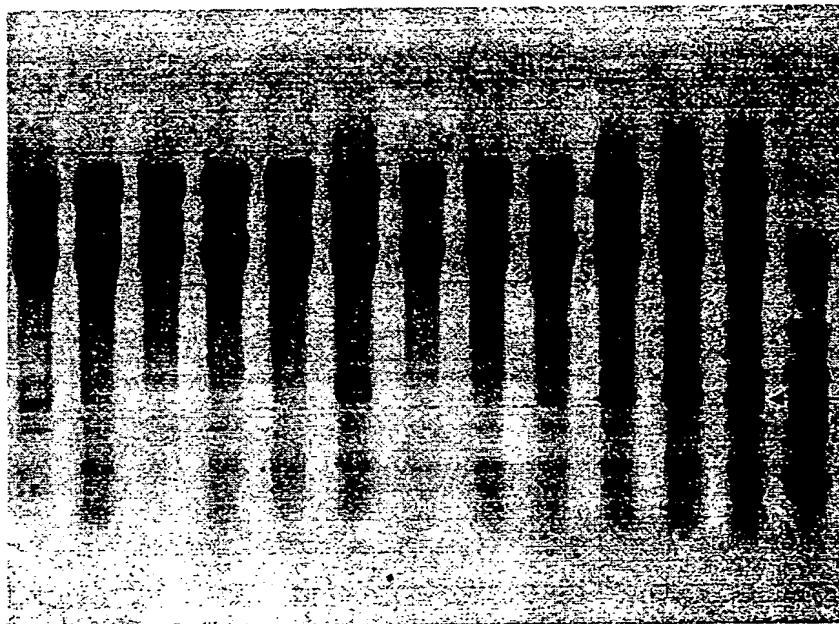
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248/254

FIGURE 61

Activation of pAP-288 protein

A B C D E F G H I J K L M



390 b
fragment

- A. 200 ng of pAP-288
- B. 28.4 ng of pAP-288
- C. 4.0 ng of pAP-288
- D. 482 pg of pAP-288
- E. 83.4 pg of pAP-288
- F. Ricin A chain
- G. Negative control
- H. 83.4 pg of pAP-288 digested with tissue Plasminogen Activator (t-PA)
- I. 482 pg of pAP-288 digested with t-PA
- J. 4.0 ng of pAP-288 digested with t-PA
- K. 28.4 ng of pAP-288 digested with t-PA
- L. 200 ng of pAP-288 digested with t-PA
- M. RNA ladder

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249/254

FIGURE 62

Cleavage of pAP 294 With Human Neutrophil Elastase



A. Ricin Standard (0.050 μ g)

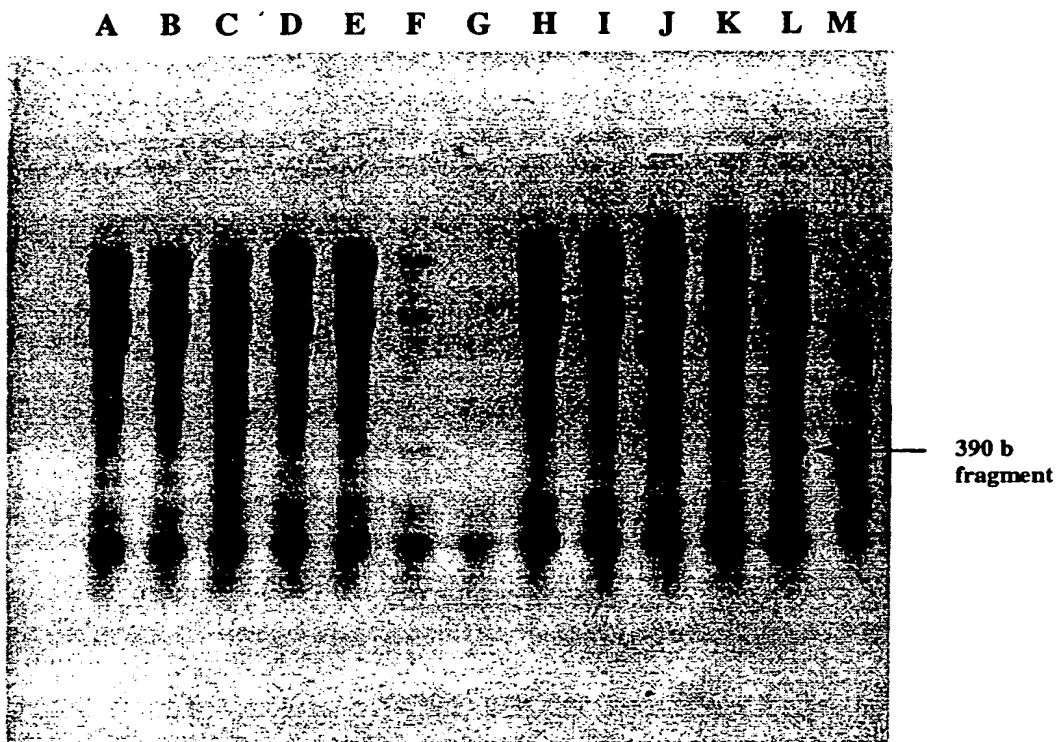
B. pAP 294 protein (0.171 μ g) digested with 1.42 μ g of Human Neutrophil Elastase

C. pAP 294 protein (0.121 μ g)

250/294

FIGURE 63

Activation of pAP 294 Protein

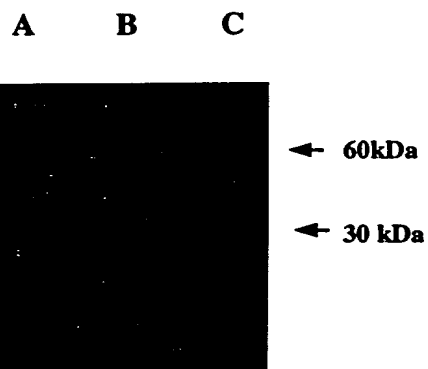


- A. 60 ng of pAP 294
- B. 8..57 ng of pAP 294
- C. 1.22 ng of pAP 294
- D. 175 pg of pAP 294
- E. 25 pg of pAP 294
- F. Ricin A chain
- G. Negative Control
- H. 360 ng of pAP 294 digested with Human Neutrophil Elastase
- I. 51 ng of pAP 294 digested with Human Neutrophil Elastase
- J. 7.3 ng of pAP 294 digested with Human Neutrophil Elastase
- K. 1.0 ng of pAP 294 digested with Human Neutrophil Elastase
- L. 150 pg of pAP 294 digested with Human Neutrophil Elastase
- M. RNA ladder

251/254

FIGURE 64

Cleavage of pAP 296 with Calpain



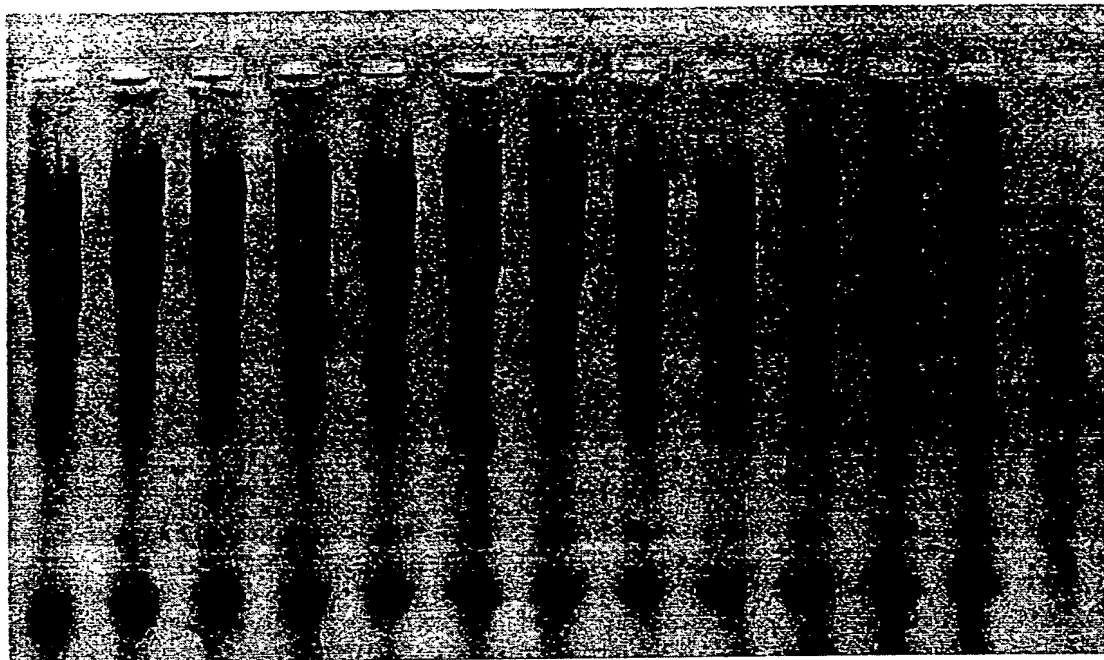
- A. Ricin Standard (0.05 µg)
B. pAP 296 (0.761 µg) undigested
C. pAP 296 (0.761 µg) digested with 4.0 µg of Calpain

004F40"TSF560

FIGURE 65

Activation of pAP 296 Protein

A B C D E F G H I J K L M



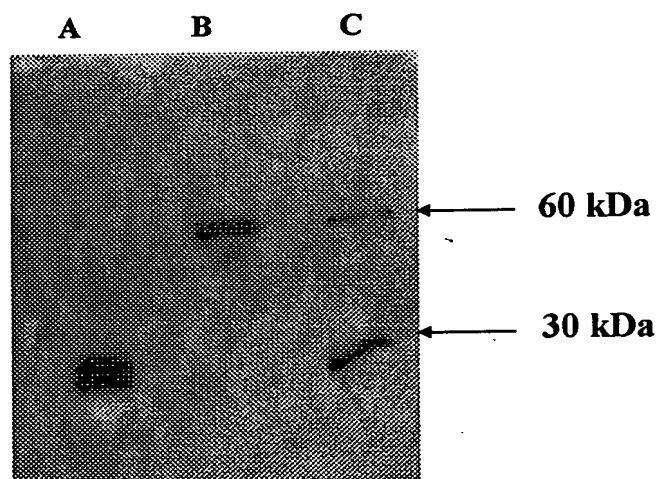
390 b
fragment

- A. 100 ng of pAP 296 variant
- B. 14.2 ng of pAP 296 variant
- C. 2.0 ng of pAP 296 variant
- D. 290 pg of pAP 296 variant
- E. 46 pg of pAP 296 variant
- F. Ricin A chain
- G. Negative control
- H. 46 pg of pAP 296 variant digested with Calpain
- I. 290 pg of pAP 296 variant digested with Calpain
- J. 2.0 ng of pAP 296 variant digested with Calpain
- K. 14.2 ng of pAP 296 variant digested with Calpain
- L. 100 ng of pAP 296 variant digested with Calpain
- M. RNA ladder

253/254

FIGURE 66

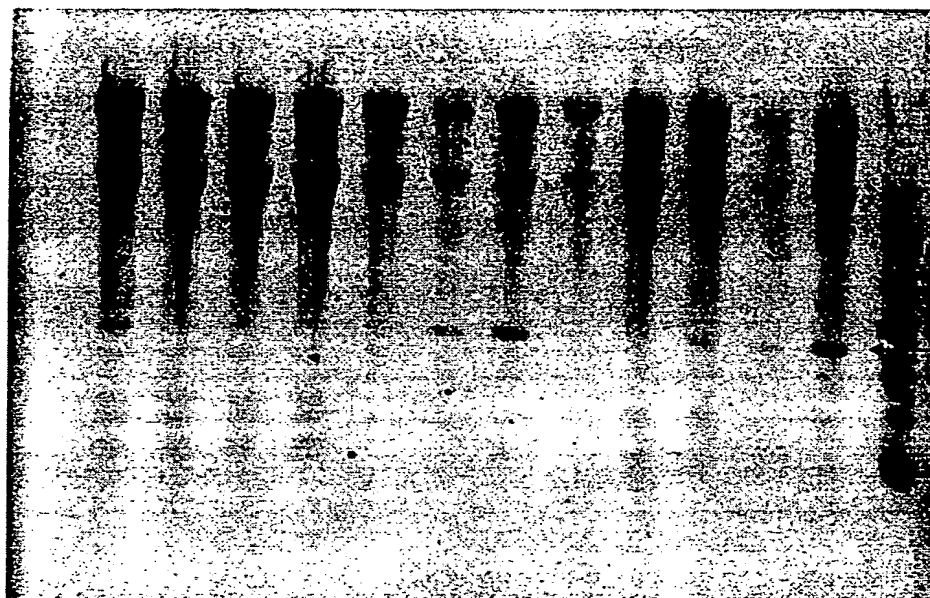
Cleavage of pAP-222 Protein by The Matrix Metalloproteinase 2 (MMP-2)



- A. Ricin Standard (0.250 ug)**
B. pAP-222 Protein (0.250 ug)
C. pAP-222 protein (0.250 ug) digested with 0.28 ug of MMP-2

FIGURE 67**Activation of pAP-222 Protein**

A B C D E F G H I J K L M



390 b fragment

- A. 100 ng of pAP-222 variant
- B. 14.2 ng of pAP-222 variant
- C. 2.0 ng of pAP-222 variant
- D. 291 pg of pAP-222 variant
- E. 41.7 pg of pAP-222 variant
- F. Ricin A chain
- G. Ricin A chain
- H. 41.7 pg of pAP-222 digested with MMP-2
- I. 291 pg of pAP-222 digested with MMP-2
- J. 2.0 ng of pAP-222 digested with MMP-2
- K. 14.2 ng of pAP-222 digested with MMP-2
- L. 100 ng of pAP-222 digested with MMP-2
- M. RNA ladder